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THE EFFECT OF HIGH-TEMPERATURE STORAGE ON THE DIGESTIBILITY OF CARBOHYDRATES IN POUCH BREAD

by

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13. ABSTRACT (Maximum 200 words)

Berkowitz and Oleksyk (Berkowitz, D., and Oleksyk, E. Leavened breads with extended shelf-life, U. S. Patent 5,059,432, Oct 22, 1991) from the Sustainability Directorate have developed a high-quality bread product, which was later shown by Hallberg (Hallberg, L. Staling of long-shelf bread as determined by thermal analysis, University Press, University of Massachusetts, Amherst, MA, 1996 pp 1-144) to undergo little or no staling during storage at 21° C for 3 years. This report is concerned with the digestibility (total digested simple sugars/total carbohydrates) by pancreatic α-amylase of the carbohydrates (CHO) in these pouch breads (PBRDs) stored at -18, 27, 38, and 49° C for 0, 1, 3, 6 & 12 months.

   A simple α-amylase digestion method, without the need for auxiliary protease and lipase enzymes, was developed for discerning subtle differences in digestibility during prolonged high temperature storage of pouch bread. Following solubilization of the CHO by autoclaving for 30 min, a 1 h incubation of ground PBRD with α-amylase resulted in 78% digestion as estimated by total simple sugars estimated by HPLC. The untreated CHO in PBRD stored at -18 and 27° C and ground but not autoclaved were digested to only 59%. After 6 months storage at 38 & 49° C, the digestibility (DIG) decreased to 53%. At the end of 12 months, the digestibility of the bread stored at 49° C dropped to 42%. The *mean* distribution of the digested components in the samples was essentially the same, irrespective of the length and temperature of storage, but was influenced by the treatment of the samples, nonautoclaved or autoclaved: glucose (27 or 21%); maltose (44 or 47%); and maltotriose (29 or 32%), respectively.
(Continued)

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Bread	Digestibility	Resistant Starch	Retrogradation	Sensory Analysis
High Temperature		Amylose	Amylopectin	High Temperature
Long-Term Storage		Staling	Acceptability	

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Pouch bread stored at -18° C irrespective of the length of storage up to 12 months; all pouch bread stored for 1 month, irrespective of the heat stress up to 49° C; pouch bread stored for 6 months at 27° C and pouch bread stored for 3 months at 38° C were considered satisfactory as determined by sensory analysis for overall quality, texture, appearance, flavor and odor; pouch bread stored at 49° C became unsatisfactory at 3 months. Pouch bread stored at 27° C for 12 months was judged to be marginally satisfactory with a rating of 4.6. However, the gelatinized starch in PBRD, presumably 100% gelatinized, remained digestible even after prolonged storage at 38° C. Supporting this was the finding that little or no increase in enzyme-resistant starch was observed in any of the heat-stressed samples stored for 12 months.

The method developed here for carbohydrate digestion was able to differentiate between retrograded amylopectin and retrograded amylose. Due to the use of sucrose polyester, little or no retrograded amylose crystallites were presumably formed during long term storage at high temperatures; hence pouch bread digestibility following gelatinization of starch was minimally affected. Good correlation was observed between sensory rating scores and the digestibility of ground bread used without autoclaving.

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THE EFFECT OF HIGH TEMPERATURE STORAGE ON THE DIGESTIBILITY OF CARBOHYDRATES IN POUCH BREAD

SUMMARY

Berkowitz and Oleksyk (Berkowitz, D., and Oleksyk, E., Leavened breads with extended shelf-life, U. S. Patent 5,059,432, Oct 22, 1991) from the Sustainability Directorate have developed a high-quality bread product, which was later shown by Hallberg (Hallberg, L. Staling of long-shelf bread as determined by thermal analysis, University Press, University of Massachusetts, Amherst, MA, 1996 pp 1-144) to undergo little or no staling during storage at 21° C for 3 years. This report is concerned with the digestibility (total digested simple sugars/total carbohydrates) by pancreatic α-amylase of the carbohydrates (CHO) in these pouch breads (PBRDs) stored at -18, 27, 38, and 49° C for 0, 1, 3, 6 & 12 months.

A simple α-amylase digestion method, without the need for auxiliary protease and lipase enzymes, was developed for discerning subtle differences in digestibility during prolonged high temperature storage of pouch bread. Following solubilization of the CHO by autoclaving for 30 min, a 1 h incubation of ground PBRD with α-amylase resulted in 78% digestion as estimated by total simple sugars estimated by HPLC. The untreated CHO in PBRD stored at -18 and 27° C and ground but not autoclaved were digested to only 59%. After 6 months storage at 38 & 49° C , the digestibility (DIG) decreased to 53%. At the end of 12 months, the digestibility of the bread stored at 49° C dropped to 42%. The *mean* distribution of the digested components in the samples was essentially the same, irrespective of the length and temperature of storage, but was influenced by the treatment of the samples, nonautoclaved or autoclaved: glucose (27 or 21%); maltose (44 or 47%); and maltotriose (29 or 32%), respectively.

Pouch bread stored at -18° C irrespective of the length of storage up to 12 months; all pouch bread stored for 1 month, irrespective of the heat stress up to 49° C; pouch bread stored for 6 months at 27° C and pouch bread stored for 3 months at 38° C were considered satisfactory as determined by sensory analysis for overall quality, texture, appearance, flavor and odor; pouch bread stored at 49° C became unsatisfactory at 3 months. Pouch bread stored at 27° C for 12 months was judged to be marginally satisfactory with a rating of 4.6. However, the gelatinized starch in PBRD, presumably 100% gelatinized, remained digestible even after prolonged storage at 38° C. Supporting this was the finding that little or no increase in enzyme-resistant starch was observed in any of the heat- stressed samples stored for 12 months.

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long-term storage at high temperatures; hence pouch bread digestibility following gelatinization of starch was minimally affected. Good correlation was observed between sensory rating scores and the digestibility of ground bread used without autoclaving.

INTRODUCTION

As a means of boosting the morale of the troops in the front lines and as a way of enhancing the acceptance of carbohydrates in MRE and T rations, Berkowitz and Oleksyk (1) from the Sustainability Directorate developed an MRE pouch bread, which has been shown to be satisfactory (2) even after three years of storage at 21°C. The successful outcome of this product was possible by the use of a combination of hurdle techniques and through the inclusion of innovative antistaling compounds (3,4). Specific data on the digestibility of carbohydrates during long-term storage of bread under low, medium and high temperatures are virtually nonexistent. The purpose of this investigation (5)[1] was to determine to what extent the digestibility of carbohydrates in pouch bread is adversely affected as a result of storage during low and high temperatures for prolonged periods. Because of the wide variety of in vitro and in vivo procedures used by other investigators in the past, an effort was also directed towards developing a simple nonanimal assay procedure to assist in answering these questions.

Carbohydrates constitute the largest proportion of metabolizable nutrients in the diet, often exceeding 45% of the total calories. The daily bread is an important source of dietary complex carbohydrates. During combat field feeding, it is difficult to either procure fresh bread or to set up field bakeries. In spite of the fact that, unlike fats and proteins, complex carbohydrates are less susceptible to chemical changes, bread does not store well at room temperatures. This fact is especially true under the expected environmental extremes of the battlefield, since it is subject to retrogradation, recrystallization, staling, firming and associated undesirable texture changes. The process of retrogradation is essentially a physical transformation brought about by the crystallization of amylopectin and amylose. Amylose is a linear glucose polymer linked by α-(1--4) glycosidic bonds (6,7). Amylose may contain about 600 glucose units and, therefore, is of much smaller size (10^5 daltons) compared with amylopectin (10^7 daltons). Amylopectin contains both α-(1--4) and α-(1--6) linkages. In

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[1] This work was presented earlier at the American Institute of Nutrition Meetings, Atlanta, GA, April 1995 (see ref 5).

plants, the starches are packaged in granules with their shapes ranging from spherical in potatoes, polyhedric in cereals, to kidney shape in legumes (7). Researchers suspect crystallized amylose to form stiff double helices between the ends of the molecules during chain elongation followed by very tight lateral association of the helical regions (8-12). Amylose crystallinity is not reversed by heating. On the other hand, amylopectin crystallization, gel stiffening and resultant staling are considered to be thermoreversible, especially at 95° C and this decrystallization has been attributed to the outermost short branches of amylopectin, which give rise to low temperature melting crystals.

Retrograded (i.e., recrystallized) starch is of concern because of its resistance to digestion by amylases. Retrogradation of starch may be brought about by a number of processes and procedures used in the preservation and preparation of foods, such as freezing, thawing, heating and freeze-drying. A number of investigators have reported on the effect of processing and preparation for serving on the formation of resistant starches in foods (10-14). For example, Englyst and Cummings (14) have evaluated the digestion of potato in humans and have reported that cooling after cooking increased resistant starch. Reheating and cooling successively further increased resistant starch (RS) at every cycle. Similarly, when raw plantain (green banana of a special variety) is cooked and cooled, the amylase RS was shown to increase by 10% by weight (15). Berry (12) observed that when wheat starch solutions were autoclaved for 45 min, the yield of RS increased in proportion to the temperature of the autoclave, i.e., from 2.5% at 100° C to 9% at 134° C.

Most of the pasta products are known to elicit low blood glucose responses (16). Similar slow rate of digestion of pasta by amylase in vitro has also been reported and is ascribed to the greater density of the product. Wet homogenized pasta, on the other hand, was well digested in vitro. Furthermore, bread baked from spaghetti ingredients was rapidly digested in vitro and in vivo (16). Using breath hydrogen as an indicator, Anderson et al. (17) concluded that even white bread was incompletely digested and incompletely absorbed in the small bowel in 17 out of 18 subjects. It has been estimated that the incompletely absorbed starch in foods may range from 2 to as high as 20%. However, not all of this malabsorbed carbohydrate is attributable to resistant starch that is actually formed during processing. For example, in white bread, it has been reported that 6% of the starch survived transit through the small intestine and that the actual resistant starch was only about 1.1%. On the basis of these studies, Englyst and Cummings (14,18) have shown that 2.5% of the starch in white bread reached the terminal illeum and was mostly resistant to α-amylase digestion. About 12% of starch from cooked and cooled potato escaped digestion in the small intestine.

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Sievert and Pomeranz (19) assessed the conditions that influence the formation of RS from starches. They found that the heating temperature, the number of heating-cooling cycles, the chemical structure of the starch, and the starch-water ratio were important. A yield of 21% was obtained with amylomaize VII starch containing 70% amylose and a yield of only 2.5% with waxy maize starch (<1% amylose and almost entirely amylopectin). By increasing the number of cycles to 20, it was possible to raise RS to as much as 40%. Comparing conventionally cooked with factory-processed foods, Brand et al. (20) reported that the digestion rate by porcine pancreatin was vastly increased in processed foods (rice bubbles, presumably a type of puffed rice, made by Kellog, or instant rice vs cooked rice; corn chips or corn flakes vs sweet corn; potato crisps or instant potato vs cooked potato). Blood glucose responses in humans were also similarly influenced by the kind of treatment of the foods. The conditions that favor gelatinization or disruption of organized granular structure of the starch, such as mechanical agitation and high temperature hydration as found in extrusion puffing, tend to enhance digestibility.

Although the salivary amylases play a role in carbohydrate digestion, the most important enzyme is the α-amylase secreted by the pancreas that reaches the intestine through the duct of Wirsung, which joins the common bile duct just before the duodenum (21). Alpha-amylase (1,4, α--D-glucan glycanohydrolase, EC 3.2.1.1) generally hydrolyzes the α-1,4- glycosidic bonds in a random fashion to maltose and maltotriose (6). With amylopectin, small oligomers less than six glucose units containing the branch α-1,6 linkage (branched α-dextrins) will also remain undigested. β-amylase (1,4-α-D-glucan maltohydrolase, EC 3.2.1.2) is of plant origin, which acts from the nonreducing end of the oligosaccharide and stops at the branch 1,6 linkage giving rise to maltose units and large polymers with multiple branches. Glucoamylase (1,4-α-D-glucan glucohydrolase, EC 3.2.1.3) has been reported only in bacteria and molds and functions by removing glucose units from the nonreducing end (6). It also hydrolyzes α-1,6 linkages but somewhat more slowly than the α-1,4 bonds. However, Gray (22) has reported that glucoamylase and amyloglucosidase are present at the intestinal brush border surface. The presence of membrane-bound glucosidases, including a glucoamylase and a sucrase α-dextrinase complex, has also been described by Anniston and Topping (7).

Alpha-1,6-glucosides, such as isomaltose, isomaltotriose and panose are hydrolyzed by the intestinal enzyme, oligo-1,6-glucosidase (dextrin 6α-D-glucanohydolase, EC 3.2.1.10). There are a number of other intestinal enzymes that have been considered important to compete the final digestion of the polysaccharides. For example, maltose is hydrolzed to glucose by α-glucosidase

(α-D-glucoside glucohydrase EC 3.2.1.20, maltase) while lactose is acted upon by a galactosidase (β-D-galactoside galactohydrolase, EC 3.2.1.2) since it recognizes the galactose residue and not the glucose residue of this disaccharide. With sucrose, two different enzymes can break the linkage between glucose and fructose. Both α-glucosidase (EC 3.2.1.20) and β-fructofuranosidase (EC 3.2.1.26) can hydrolyze sucrose because the first enzyme structurally fits glucose but the second enzyme recognizes fructose.

RESISTANT STARCH

Carbohydrate foods that are resistant to amylolytic digestion may be due to a variety of causes such as the presence of intact starch granules, the physical matrix of the foods, encapsulation or coating of the carbohydrate entity by other food components, and retrogradation of the amylopectin or of the amylose moiety. Englyst et al. (23,24) have suggested an in vitro method for the nutritional classification of starch: first, starch is divided into slowly digestible (SDS) and rapidly digestible fractions (RDS). The portion that is undigestible is broadly grouped as the resistant starch (RS) and is, in turn, divided into physically inaccessible starch (RS1); resistant starch granules and retrograded amylopectin (RS2); and retrograded starch (retrograded amylose, RS3, 24). The digestion methods that have been suggested include a variety of enzymes: α-amylase, heat stable Teramyl (a heat stable bacterial α-amylase, marketed by Novo Laboratories, Inc., Wilton, CT) and a fungal amyloglucosidase, as well as protease, lipase, etc. While there may be justification to use the fat and protein splitting enzymes, the use of fungal enzymes at low pH (4.6 or 5.2) and at high temperatures to model human digestion is questionable. It is possible that the fungal amyloglucosidase is similar to the membrane-bound glucoamylase reported by Annison and Topping (7), but no comparative data have been reported. It has been suggested (Englyst et al., 24) that the data obtained by using the combination of these enzymes is comparable to that obtained under physiological conditions, but no published data or articles are available.

The method used by previous investigators (12, 23, 24) has been described in detail. Between 1-4 g of the dried and finely milled samples of the foods (0.2 mm diameter) are incubated with pepsin at a pH of 1.3 to mimic gastric digestion. Glass beads are used to break up the food particles. Then a sufficient volume of 0.25 M sodium acetate is added to bring the pH to 5.2 and incubated with an enzyme mix (pancreatin, amyloglucosidase and invertase), at 37° C with shaking. Pancreatin is a mixture of several enzymes: amylase, protease and lipase. At the

end of 20 min, and 120 min, 0.5 mL samples are withdrawn and added separately to 20 mL of 66% ethanol and analyzed for glucose. The remainder of the solution is vigorously boiled 30 min at 100° C, cooled to 0° C and 7 M KOH added and mixed for 30 min at 0° C with shaking. One mL is taken and adjusted to pH 5.2 and incubated at 70 to 100° C with amylogluocosidase, cooled and assayed for glucose.

Proksy's et al.'s method (25) for the determination of total dietary fiber utilizes the heat stable α-amylase, Teramyl, to gelatinize the starch as well as a protease to digest the protein and amyloglucosidase to digest the dietary carbohydrates. The residue left is estimated as the total dietary fiber. Resistant starch should theoretically be present in this fraction providing it is a tightly bound retrograded amylose crystallite.

CRYSTALEAN AND NOVELOSE

There are two commercial starches on the market that are claimed to contain about 30% of starch that are resistant to amylase digestion. Crystalean (9), which is marketed by OPTA FOOD Ingredients, Inc., is covered by US patent 5,051,271 dated Sep 1991. This product is claimed to be a food-grade bulking agent to be used as a texturizing compond in low-fat food formulations such as in sugar cookies, microwavable fudge, 30% fat margarine, reduced-flour brownies and in nonfat frozen dairy dessert. It has been suggested that levels up to 20% can be incorporated without adverse effects upon texture or flavor. This product contains retrograded amylose crystallites, which are highly resistant to amylase digestion. According to the data (9), it is also resistant to digestion by Teramyl and by fungal amyloglucosidase.

An early study by Anderson et al. (17) using breath hydrogen data, suggested that 2% to 20 % of the carbohydrates in white bread and in macaroni were not absorbed in humans. Bread made from low gluten wheat, from gluten plus low gluten wheat, or rice bread did not result in increase in breath hydrogen, suggesting that gluten was not the cause of altered breath hydrogen levels in humans. Snow and O'Dea (13) demonstrated that two commercial breads were digested by α-amylase and amyloglucosidase in 30 min to 80% compared with only 61% for homemade white bread. Englyst et al. (26) estimated RS in several cereal foods and bread and observed them to be in the range of 0.5 to 3.0%. Based upon blood glucose levels following food ingestion, Granfeldt and Bjorck (16) showed spaghetti produced the lowest glucose response of 60% compared with porridge prepared from spaghetti or a bread baked from spaghetti

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ingredients (73 and 100% respectively). They concluded that the special texture of spaghetti was vital in flattening the blood glucose response curve.

Granfeldt et al. (27) prepared two types of corn-based bread, known as arepas which are widely used in Columbia and Venezuela. One bread is prepared with dent corn flour (25% amylose), while the other bread is prepared with high amylose corn flour (70% amylose). Both flour dispersions in water were subjected to four heating-cooling cycles to maximize the amount of RS. The goal was to evaluate RS concentration in vitro using Teramyl, pepsin and pancreatin and in vivo using rats treated with the antibiotic, nebacitin, to suppress hindgut fermentation. In rats, the low and high amylose corn breads were digested in the small intestine to 96 and 68% of the starch, respectively. Further, there was greater fermentation (63%) of the RS in rats fed dent corn bread as opposed to only 11% in rats fed the high amylose corn bread. A review of the literature revealed the many methods for assessing the digestibility of carbohydrates in use and the difficulties in obtaining definitive data on the factors influencing the formation of resistant starch (retrograded amylopectin and retrograded amylose) in food products under normal and stressful conditions and how these factors impact upon digestibility.

The specific objectives of this study were to a) standardize a simple nonanimal assay procedure for estimating the digestibility of carbohydrates in starchy foods; b) determine the effect of prolonged storage at several temperatures on the digestibility of pouch bread; and c) attempt to define how staling and retrogradation of pouch bread influences the relative digestibility of the linear and branched chain glucose polymers.

MATERIALS AND METHODS

MRE POUCH BREAD

The ingredients used for the pouch bread are given below. The dough was prepared by the standard dough method and was divided into portions of 75 g each and baked in a rotary oven at 350° C for 20 to 25 minutes (28). Upon cooling to about 38° C, each single portion was inserted into trilaminate pouches and sealed under vacuum. An oxygen-absorbing packet was used to reduce oxygen tension to less than 0.01%. The bread was stored at -18°, 27°, 38° and 49° C for 1, 3, 6, and 12 months and stored at -18° C prior to analysis.

CHEMICAL ANALYSIS

Proximate analyses. Moisture, fat, protein and ash were estimated using standard AOAC procedures (29). Glucose assays were conducted using the enzymatic procedure with reagents purchased from Sigma Chemical Co. (St. Louis). Glucose is converted to glucose-6-phosphate by hexokinase. In an enzyme-coupled reaction, glucose-6-phosphate is converted to 6-phosphogluconate with the concurrent reduction of NAD (Nicotine adenine nucleotide) to NADPH (reduced nicotine adenine nucleotide) . The formation of NADPH results in an increase in absorption at 340 nm, which is directly proportional to the glucose concentration.

Total reducing sugar analysis. Glucose, maltose maltotriose and other reducing sugars were estimated using the alkaline 3,5-dinitrosalicyclic acid (DNSA) reagent. This reagent is frequently used for the estimation of reducing substances in biological fluids. The reagent was prepared by dissolving 2.5 g of DNSA in 50 mL of 2N NaOH in a 500 mL beaker during heating on a hot plate. In a separate 250 mL beaker, 75 g of sodium potassium tartrate was dissolved in 125 mL of Milli Q (deionized water of high purity, conductivity, 17 megohms , Millipore Co., Bedford, MA) and added to the DNSA solution and made to 1000 mL.

Phosphate buffer, pH 6.9. Potassium dihydrogen phosphate, KH_2PO_4, 6.05g, Sodium monohydrogen phosphate, Na_2HPO_4, $7H_2O$,12.2g and sodium chloride 0.8 g (Fisher Scientific Co (Springfield, MA) were all dissolved in 1L of Milli Q and the pH was adjusted, if necessary, to 6.9 with small volumes of either 10% phoshoric acid or 1N NaOH. This buffer was used for pancreatic α-amylase digestion of pouch bread samples.

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Acetate buffer, pH 4.6.   Two hundred and fifty mL of 0.2N acetic acid (10.92 g/1L Milli Q) was added to 250 mL of 0.2N sodium acetate, CH_3COONa, $3H_2O$ (27.2 g/1L) and additional Milli Q was added to bring the volume to about 950 mL. The pH was adjusted to 4.6 with the required addition of the acid or the salt solution and the volume made up to 1L. This buffer was used in digestion experiments using amyloglucosidase.

PRETREATMENT OF THE SAMPLES PRIOR TO ENZYMATIC DIGESTION

Grinding. Each pouch bread was sliced and cut into horizontal and vertical strips to obtain small pieces of approximately 1 cm^3. The bread pieces were transferred to one-pint Mason jars and blended in a Waring blender. During the grinding operation, the jars together with the blenders were held tight and shaken to redistribute the particles at the bottom and the top. This shaking produced a homogenous product of fairly small particle size. Due to the presence of emulsifiers and glycerol, it was not possible to obtain fine particles. The grinding time is critical; excessive grinding may result in a gummy product due to overheating.

Polytron Homogenization. One hundred to two hundred mg of the bread samples were dispersed in 8 mL of the incubation buffer in pyrex test tubes (16 mm OD x 12mm L) and homogenized in a Kinematica (Polytron) homogenizer (Luzern, Switzerland) for 2 min. The homogenates were transferred to 25 mL polycarbonate Erlenmeyer flasks along with two 1 mL rinses and digested (see below) with either α-amylase or fungal amyloglucosidase.

Autoclaving. One to two hundred mg of ground pouch bread samples were dispersed in 9 mL of the phosphate buffer, pH 6.9 and autoclaved for 30 min at 121° C.

ENZYME PREPARATIONS

Alpha-amylase. Fifty mg of α-amylase (20 units/mg, Sigma Chemical Co., St Louis, MO) were carefully homogenized in 25 mL of the phosphate buffer, pH 6.9 using a Mixxor glass homogenizer (Lidex Technologies, Bedford, MA).

Fungal (Aspergillus niger) amyloglucosidase. This preparation is in 1M glucose solution (Sigma Chemical Co., St Louis, MO). Therefore, blank corrections for

glucose are essential. This enzyme was used without dilution. Typically, the enzyme volume used was 25 µL.

ENZYMATIC DIGESTION OF CARBOHYDRATES

Alpha-amylase. In order to display a well dispersed substrate to the enzyme, pancreatic α-amylase, small amounts (0.1000 g) of the bread, ground well in blender were dispersed in 9 mL of 0.05M phosphate buffer, pH 6.9 and autoclaved for 30 min at 121.1° C. Upon cooling to ambient temperature, 1 mL of the homogenized enzyme (2 mg/mL, Sigma Chemical Co., 20 U/mg) was added and incubated for 1 h at 38° C in Dubnoff shaking water bath in 25 mL polycarbonate flasks. At the end of the incubation, the solutions were cooled on crushed ice, centrifuged and filtered through Milex filters (Waters, 25mm dia, 0.45 µm pore size) and analyzed by HPLC using a Waters carbohydrate column with 70% acetonitrile-water (flow rate of 2 mL/min) as the mobile phase. The detection and quantification was with a Waters Refractive Index Detector. Six replicate samples from each treatment group were analyzed at one time. In a variation of this method, the ground pouch bread was dispersed in phosphate buffer and was incubated with α-amylase without prior autoclaving.

Amyloglucosidase digestion. This was conducted with 100 to 200 mg of the bread samples in 10 mL of acetate buffer, pH 4.6 along with 25 µL of amyloglucosidase solution. The pretreatment for the samples included polytron homogenization. Digested samples were withdrawn from the incubation flasks at regular intervals, typically 2, 5, 10, 20, 120 min and sometimes 24 h and iced. Separate flasks were used for each time period to enable withdrawal of multiple samples at one time. Aliquots, usually 4 mL, were adjusted to pH of 7 and the volumes were then made up to 10 mL and analyzed for glucose by the hexokinase method.

PHYSICAL MEASUREMENTS.

HPLC Analysis. A Waters Liquid Chromatographic system (Waters Associates, Milford, MA) with a refractive index detector, a Waters carbohydrate column, an autosampler, a system integration module (SIM box) and an 840 computer system were used. The SIM box converted the analog signals from the detector to digital signals and enabled the quantification of the digested sugars, glucose, maltose and maltotriose.

<u>Instrumental Texture Measurement.</u> The texture of the bread samples was analyzed with a Voland-Stevens-LFRA texture analyzer. A 61 mm polycarbonate blade was attached to the plunger and the rate of travel by the blade was 2 mm/s with the depth of penetration being 10 mm. The peak resistance to penetration was recorded in triplicate per sample.

SENSORY ANALYSIS

Sensory analysis for appearance, flavor, odor, texture and overall quality was conducted with a trained technical quality scoring panel and evaluated on the basis of one (extremely poor) through nine (excellent) rating scale. Samples falling below a rating of 4 were deemed unsatisfactory and were not evaluated. However, for the purpose of plotting of data, texture scores and overall quality of sample that were considered unsatisfactory were arbitrarily assigned a score of 3. It was presumed that the ratings would likely have been much lower in many cases than the assigned value of 3. However, this assignment of the score of 3 is totally subjective and is open to challenge.

STATISTICAL ANALYSIS

One way and two way Analysis of Variance (ANOVA) were used to test (30) the effect of storage time, storage temperature or both. There were 4 temperatures (-18°, 27°, 38° and 49°C) and 4 storage periods (1, 3, 6, and 12 months). There were 6 replicates for each treatment; thus, there were a total of 96 (4x4x6) individual samples. If the ANOVA tests were significant, multiple range tests were conducted.

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COMPOSITION OF POUCH BREAD

Ingredient	Percent by weight
Flour	50.53
Water	28.96
Shortening	8.50
Glycerol	6.34
Yeast	2.25
Salt	1.29
Emulsifier*	1.00
Gum arabic	0.50
Calcium sulfate	0.25
Xantham gum	0.25
Encapsulated sorbic acid	0.10
Cream flavor	0.03

*Sucrose ester stearate

RESULTS

A Waters carbohydrate column, 80% acetonitrile- 20% water mobile phase at 2 mL/min flow rate, and refractive index detection are widely used for the chromatographic separation of simple sugars in food products. However, even if one of the products to be analyzed is a trisaccharide such as maltotriose or higher oligomers and interfering compounds, the 80% acetonitrile system is not practical since it takes an inordinate amount of time to elute these compounds (ca. 12 to 25 min). Therefore, we investigated a number of mobile phases and found that 70% acetonitrile was a much better choice. The resolution of glucose, maltose and maltotriose standards obtained with this mobile phase is shown in Figure 1. Enzymatic digests are not as clean as sugar extracts of foods and, therefore, are not as well resolved as the standards (Figure 2). It is to be noted that the skewed peak seen at 9 to 10 min has approximately the same retention time (RT) as maltohexose (Figure 3). Maltoheptose has a retention time of about 14 to 15 min. However, our subsequent work has demonstrated that phosphate anion gives rise to this skewed peak with an RT between 9 and 10 min. Further, the absence of any large oligomers in bread digests was confirmed using a Sugar Pak I column obtained from Waters Associates.

The pouch bread samples, which were ground in the blender and autoclaved in the incubation buffer, were digested by pancreatic α-amylase in 1 h under the present experimental conditions to an average of 78%. The percentage of carbohydrate digested was calculated as: (sum of enzyme digested glucose, maltose and maltotriose in the sample determined by HPLC) x 100/ (total carbohydrate in the same amount of the sample). The percent total carbohydrates was calculated from proximate analysis: [100-(protein + fat + moisture + ash)]. The data (Figure 4) presented in the bar graphs show that the digestibility is not substantially affected during each withdrawal period by the extremes of the storage temperature ranging from -18 to 49°C. One way ANOVA demonstrated that the digestibility of the 12-month samples at 49°C were significantly different ($P<0.003$) than others during this withdrawal period.

With the nonautoclaved samples, the average digestibility (Figure 5) decreased to an average of 55% and this fact is possibly due to thermolabile starch particles, which are resistant to digestion at 37° C (see discussion),

as well as the difficulty in presenting uniform size particles to the enzyme. In general, there was a trend towards lower digestion values in the bread stored at 38 and 49°C compared with bread stored at -18°C. Additionally, the bread stored for 12 months was less digested. Two-way ANOVA indicated significant effects due to time and temperature. At almost all withdrawal periods (except during 3 month and the 27°C sample at 6 month withdrawal), samples stored at 27, 38 and 49°C were significantly ($P<0.05$) less digested than the control samples stored at -18°C. For the samples stored for 3 months, only the digestibility for samples at 49°C storage was significantly lower ($P<0.05$) than the control sample.

There may be a hazard in placing reliance on HPLC-generated data alone. In this case, for example, all digestion data were derived from peak areas of sugar components resolved by HPLC. Furthermore, with enzymatic digestion, sufficient base line resolution is not always possible; in addition, there is some overlap between peaks due the presence of isomers, e.g., maltose and isomaltose. For this reason, it was felt necessary to confirm part of the data by determining total reducing sugars, glucose, maltose and maltotriose by direct chemical analysis. The results of this analysis with 1 and 12 month storage sample digests are shown in Figures 6 and 7. It is seen that the percent digestion is nearly the same as that obtained with HPLC analysis. When ground samples were used, the digestion was much lower (around 60%) as opposed to ground and autoclaved samples (around 78%). Secondly, the 12 month samples, which were ground and not autoclaved, indicated a sharp decrease in digestibility with time in contrast to the autoclaved samples (Figures 6 and 7). These data obtained by direct chemical analysis support the conclusions of the HPLC analysis.

In another approach, fungal amyloglucosidase was used in an attempt to see whether the digestion rate curves would reveal substantial differences in pouch bread stored for either prolonged periods or stressed at high temperatures. The digestion data have been plotted in two ways: one, as affected by storage temperature at various withdrawal periods (Figure 8) and the other as influenced by the storage period at various temperatures (Figure 9). One plot displays how the extremes of storage temperature affect digestibility during each withdrawal. The other plot depicts the effect of a single temperature during storage of the bread over a 1 to 12 month period. The 12 month samples are digested to a lesser extent at 27, 38 and 49°C than those at -18°C (Figure 9). The rapidly digestible starch (RDS) is digested in 20 minutes and there are no significant differences in the initial rates of the reaction during the four withdrawal periods. Even the slowly digestible starch (SDS) fraction, digested in 120 min, is not influenced by storage. In the case of Figure 9, there is a trend towards a slower

rate of reaction of the SDS fraction in the sample during withdrawals at 6 and 12 months compared with the initial periods.

The composition of the digested carbohydrates was fairly constant, especially in the autoclaved samples. In the case of the autoclaved samples, the average percent composition of the digested sugars was: glucose 21 ± 3.5; maltose, 47 ± 3.1; maltotriose, 32 ± 1.8. For the nonautoclaved samples, the average percent composition of the digested sugars was: glucose, 27 ± 5.5, maltose, 44 ± 3.6; maltotriose, 29 ± 4.0. The lower value for glucose in the autoclaved samples compared with the nonautoclaved samples may be due to greater loss of glucose during nonenzymatic browning reaction at the high temperature. Representative data for 1 and 3 months at the 4 temperatures using the nonautoclaved and autoclaved samples are shown in Figures 10, 11, 12, and 13.

The presence of significant amounts of glucose in the digests is puzzling since amylase is known to hydrolyze starch to mostly maltose, maltotriose and higher oligosaccharides. It has been reported that α-amylase digestion of starch yields maltose, maltotriose and branched α-dextrins (6,22), while others have reported that glucose is also a digestion product (7). In our experience with pouch bread and with starches, we have observed glucose to be one of the digestion products. The glucose was identified both by retention time on a Waters carbohydrate column as well as with a Waters Sugar Pak I column and by an enzymatic assay specific for glucose using phosphate-6-phosphate dehydrogenase. The amylase used was a hog pancreatic α-amylase bought from Sigma Chemicals Co. It is possible that it was contaminated with other enzymes, especially dissacharidases, such as maltase.

In contrast to the data on digestibility, the sensory analysis (Figures 14, 15) indicated a pronounced decrease in the overall quality and texture as a result of the storage temperature. Similar data were obtained for the other sensory attributes, namely, appearance, flavor and odor. At 1 month storage, all samples had ratings 5 or above. This was uniformly the case with all samples stored at -18 and 27° C, irrespective of the length of storage up to 6 months; samples stored at 27° C for 12 months had average ratings of 4.7 or higher and may be considered as only marginally satisfactory. Bread stored at 38° C was rated below 5 at 6 months and below 4 at the end of 12 months. Samples stored at 49° C were rated 3 or lower at the end of 3 months and beyond. The regression lines help to emphasize these observations (Figures 16, 17). The regression fit of the data is fairly good for all periods, 1, 3, 6 and 12 months with r^2 values 0.86, 0.61, 0.71 and 0.86, respectively, for overall quality and 0.63, 0.43, 0.62 and 0.82, respectively for texture. The slopes decrease from almost zero (-0.01) to -0.046

from Month 1 to Month 3 and beyond, providing an indication of the rapid decline in ratings.

The regression charts using breads which were autoclaved suggest no decrease in digestibility over a 6 month storage period. There is a small decrease (6 %) in the 12 month samples as a result of increase in the storage temperature. One way ANOVA confirmed these observations. Interestingly, the slopes of the regression lines were 0.04, 0.011, 0.02 and -0.087 with only the 12-month plot showing a negative slope (Figure 18). With the nonautoclaved samples, there is a clear contrast. There appears to be a definite effect of temperature irrespective of the duration of storage. The slopes were with -0.08, -0.11, -0.15, and -0.15. with all the slopes being negative. The fit was poor to fair with r^2's of 0.48, 0.24, 0.64 and 0.41 respectively for the 1, 3, 6 and 12 month withdrawals (Figure. 19). The two way ANOVA indicated that both time and temperature affected digestibility ($p < 0.001$) especially the extremes of storage conditions (both 12 months and 49° C). In linear regression, since the terminal data points have a major impact, the slopes are expected to be heavily influenced.

An attempt was made to determine whether there was any correlation between the sensory texture scores and either the instrumental texture measurements or the digestibility data obtained with the autoclaved and nonautoclaved (ground only) samples.

For reasons unclear at this time, the Voland-Stevens texture measurements did not correlate with the sensory texture scores. A reason offered by one reviewer (LH) was that the instrumental texture method provides a linear response with spongy, resilient crumb but attains a maximum when the crumb becomes dense and, therefore, becomes essentially insensitive. The regression fit was poor, with the r^2 values being 0.012, 0.074, and 0.19 respectively (Figure 20). On the other hand, the correlations were quite good between the texture score or overall quality and the digestibility of ground nonautoclaved pouch bread, irrespective of the time-temperature combination, that was selected, i.e., whether the data were based upon a single temperature for 1, 3, 6 and 12 month (Figures 21 and 22) or whether they were obtained at a single withdrawal period at the four temperatures (Figures 23 and 24). The r^2 values for the nonautoclaved samples ranged from 0.55 to 0.88. For the autoclaved samples, the fit was very poor (r^2 = 0.006 to 0.06). The fit was better when the data were based upon timed withdrawals (r^2 = 0.14 to 0.69); however, the slopes were much smaller than with the nonautoclaved samples (-3.4 to 1.6 compared with 2.6 to 5.3).

Data obtained (Figure 25) with a commercial starch fiber product have demonstrated that the current method using the autoclaving procedure does not either adversely or favorably influence the extent of the retrograded amylose present in the product (observed 35%, reported 30%). It is recognized that retrograded amylopectin is thermolabile; hence autoclaving or any other heat treatment is expected to increase its digestibility.

Starch that is considered resistant to amylase digestion is usually determined by a combination of pepsin, pancreatin (amylase, protease and lipase) and a fungal amyloglucosidase. Resistant starch was estimated by the method of Prosky et al. (25) using Teramyl, a bacterial protease and fungal amyloglucosidase on single pooled sample of pouch breads stored for 12 months at -18°, 27°, 38° and 49° C. The data (Figure 26) demonstrated that resistant starch, as defined in the literature, and estimated from total dietary fiber assays, is not enhanced in pouch bread subjected to either low or high temperature storage for 12 months. Further, the value obtained (3%) is surprisingly close to the literature value of 3.1% for fiber in whole wheat flour (25). While this method is widely used, it has to be stressed that since two fungal enzymes and high temperatures are used, they may not mimic the exact situation that prevails in the human intestine. However, these data support the finding that gelatinized starch in pouch bread remained digestible even after prolonged storage (12 months) at 38° C.

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DISCUSSION

We have established by using Crystalean, a retrograded amylose product, that retrograded amylose is not digested by α-amylase irrespective of whether it is heated as a dispersion in the autoclave or not. When the pouch bread is autoclaved in a dispersed state, the effect of prolonged storage and the effect of heat stress was on RS apparently nullified. This would not have happened if retrograded amylose had been a product. As a result of prolonged storage and as a result of heat stress, the digestibility is affected in nonautoclaved pouch bread. The difference in digestibility between the initial -18°C control and the stored breads is due to heat-labile retrograded amylopectin. The question that needs to be posed is whether this portion will be or can be digested in the human alimentary tract. Based upon existing literature, it would appear unlikely that this retrograded product would be digested. On the other hand, it may be argued that no resistant starch, as determined by the Prosky's method for nondigestible fiber, was observed in the heat stressed, 12-month pouch bread samples and, therefore, the bread should be well digested.

It has to be pointed out that the Prosky's method involves considerable heating: 20 min at 95° C; and 60 min at 55° C and the concerted enzymatic attack by Teramyl, a heat-stable bacterial amylase, a bacterial protease and a fungal amyloglucosidase — conditions that do not prevail in the human intestine. The amylase undigestible fraction was estimated to be about 7 to 21%, based upon the -18° C control nonautoclaved samples. Studies in vivo with time and temperature-stressed pouch bread using methods similar to those of Englyst et al. (14, 15) with starchy foods may help to clarify this issue.

The digestion and absorption in the small intestine of dietary carbohydrates is difficult to measure in humans and animals because they may partly escape and be fermented in the colon. Thus, a number of carbohydrate digestion studies have relied upon ileostomy subjects who had total colectomies and had the terminal 10 cm of the terminal ileum removed for ulcerative colitis (14, 18, 31). Some of the enzyme assay methods used previously have been unduly complex. For example, multiple enzymes (protease, lipase, amylase and amyloglucosidase, etc.) at several pH have been used. Some investigators have used elaborate sample preparation procedures such as mastication by volunteers prior to in vitro incubation with enzymes (31). The method used here is simple and straightforward; however, its application to a wide variety of foods is yet to be demonstrated.

The fact that we have been able to use this method extensively for bread without the need for a protease or a lipase suggests that these enzymes may not be routinely required for many foods. We have used this method to determine amylase digestibility of foods, such as breakfast cereals (rice krispies, rice chex), instant rice, cooked rice, and several kinds of cooked pasta. It is conceivable that starch coated with hard fats, with protein or with inedible substances (such as shellac, chitosan, etc.) may have different effects. However, for routine analysis of starchy foods, amylase, alone or in combination with amyloglucosidase, appears to be sufficient without auxiliary boost from lipase and proteases.

The method of Englyst et al. (23,24) has been described in some detail earlier. While it is undoubtedly an all-inclusive procedure, it is much too cumbersome. For many purposes, our method is much simpler and may be adequate for many situations. Also, it provides an easy distinction between retrograded amylose and retrograded amylopectin. The procedure of Englyst et al. (23, 24) requires prior digestion with pepsin at a pH of about 1.3 followed by incubation with pancreatin, amyloglucosidase and invertase at a pH of 5.2 for 20 minutes and 120 min to obtain the RDS (rapidly digestible starch) and SDS (slowly digestible starch) and RS (resistant starch). RS could result from starch that is physically inaccessible (partially milled grains and cereals), due to resistant starch granules (raw potato or raw plantain) or due to retrograded starch (bread, cooled cooked potato, etc.). It needs to be emphasized that pancreatin contains amylase, protease, lipase and possibly other enzymes.

Distinct from the melting of crystalline solids, the phase transition that occurs when amorphous solids are transformed to the viscous liquid state is known as the glass transition. The start or the midpoint of this phase change has been considered as the glass transition temperature, T_g. However, it is currently thought to be more accurately represented as a range. Through manipulation of T_g, it may be possible to inhibit or accelerate staling of bread which is associated with retrogradation or recrystallization of amylopectin. Above T_g, the product is more elastic and less brittle. Compounds that depress T_g, such as plasticizers, cause the product to remain in a rubbery state at lower temperatures. The glass transition temperature, T_g, plays an important role in crystal nucleation and crystal growth (Morris, 8). Amylopectin crystallization is inhibited by raising T_g such as by adding sugars or glycerol, which is the case here. Amylose crystallization is a more difficult problem to arrest. The use of surfactants and amylose complexing agents, such as sucrose polyester, in this bread appears to play a role in decreasing irreversible staling in this ration item under reasonable storage conditions for prolonged periods. The mechanism of this interaction leading to desirable effects is yet to be elucidated.

In an extensive review of the literature, Morris (8) has concluded that retrograded amylopectin is thermoreversible, especially at 95° C, whereas retrograded amylose is not, which is in accord with the observation of Englyst et al. (23, 24).

On the basis of the present data on pouch bread, it is speculated here that the treatment of the autoclaved and nonautoclaved starch samples provides a basis for developing a simple enzymatic method to differentiate between retrograded amylopectin and retrograded amylose. In support of this, the experiment conducted with the commercial starch fiber containing 30% retrograded amylose demonstrated that this product is unaffected by the autoclaving procedure. Further, Sievert and Pomeranz (19) have shown that the yield of resistant starch brought about by an autoclaving treatment (1 h at 134° C) was highest (21%) with Amylomaize VII (70% amylose) and progressively decreased as the starch type used had lower levels of amylose and correspondingly higher levels of amylopectin. With waxy starch (1% amylose and about 99% amylopectin), the yield was 2.5%. Berry (12) has shown that very little RS is produced in autoclaved waxy maize starch (0% amylose) compared with Hylon V (50% amylose) where 28% RS was formed. Further, Berry has demonstrated that very low yields of RS (0.2 to 4%) were obtained with native amylopectin starches as opposed to high yields (32 to 46%) of RS with the same starches after incubation with a α-(1-->6) debranching enzyme (pullulanase). These results clearly show that the branches are effective in greatly minimizing the retrogradation of amylopectin.

Among the physiological effects of resistant starches may be mentioned the two- to three-fold increase in polymerized glucose in ileal digesta and in feces in rats fed retrograded amylose starch compared with rats fed cooked normal starch containing 24 and 2.8% retrograded starch, respectively (Schulz, et al. (32)). However, after 13 days on the diets, the rats' food intake and the body weight gains were almost identical in all groups. Similar results on the lack of effect of RS upon rat food intake and body weight gain during a three-week feeding study have been reported by De Deckere et al. (33). Dietary-resistant starch fed to rats decreased serum total cholesterol and serum triacyglycerols in two weeks.

The previous literature on feed intake and body weight gain of weanling rats fed normal and retrograded starch diets has suggested that retrograded amylose is efficiently utilized by rats. It has been suspected that retrograded amylose is utilized in the colon by microorganisms and converted to short chain fatty acids. Similar situations might prevail in humans. However, in a recent study, Ranhotra et al. (34) have reported lower feed efficiency and lower carcass fat, lower

protein, lower glycogen and lower water contents in rats fed resistant starches compared with a pregelatinized starch. They have concluded that the resistant starch provides no energy, even though a third of it was fermented in the colon.

Finally, it should be emphasized that the autoclaved bread samples showed very few significant differences in digestibility as a consequence of increasing the storage temperature or prolonging the duration of storage. With the nonautoclaved samples, the situation is exactly the opposite and appears to correlate with the data from the technical panel. Therefore, future studies hold the promise of predicting digestibilities from the ratings of the technical panels.

CONCLUSIONS

1. A simple digestion method was developed using pancreatic α-amylase without the need for auxiliary enzymes, such as protease, lipase or fungal amylases, and was shown to be readily applicable for pouch bread stored under extreme conditions simulating wartime environments.

2. The pretreatment of the samples (grinding vs grinding and heating) was shown to influence the digestibility of pouch bread.

3. When pouch bread samples were gelatinized by autoclaving, the digestibility was unaffected by either the temperature or the length of storage.

4. The digestibility of samples which were merely ground, was shown to be affected by the time and temperature of storage.

5. Sensory analysis with a Technical Panel indicated that the pouch bread stored at 27°C was satisfactory at the end of 6 months and was judged to be only marginally satisfactory at 12 months (rating of 4.6); those samples stored at 38° and 49° C became unsatisfactory at the end of 6 and 3 months respectively.

6. Correlation between digestibility and technical panel rating was observed with nonautoclaved samples, but not with autoclaved samples.

7. The heat treatment of the bread samples appears to provide a basis for discriminating between thermolabile and thermoresistant starch.

8. A commercial starch fiber product validated the thermoresistance of retrograded amylose during autoclaving.

RECOMMENDATIONS

1.   Evaluate the influence of several levels of sucrose polyester on sensory attributes and on pancreatic α-amylase digestibility of pouch bread stored for 1 to 12 months at high temperatures, -18 to 49° C.

2.   Conduct digestibility experiments with pouch bread and other starchy foods to provide a critical comparative evaluation of the present method with that of Englyst et al. (23,24).

3.   Bake bread using flour such as Amioca waxy maize starch, if possible, containing very high level of amylopectin, heat stress it for prolonged periods and obtain sensory ratings, predict α-amylase digestibilities and compare with observed analytical values.

4.   Determine the effect of heat-stressed commercial white bread upon sensory values and digestibility.

FIGURES
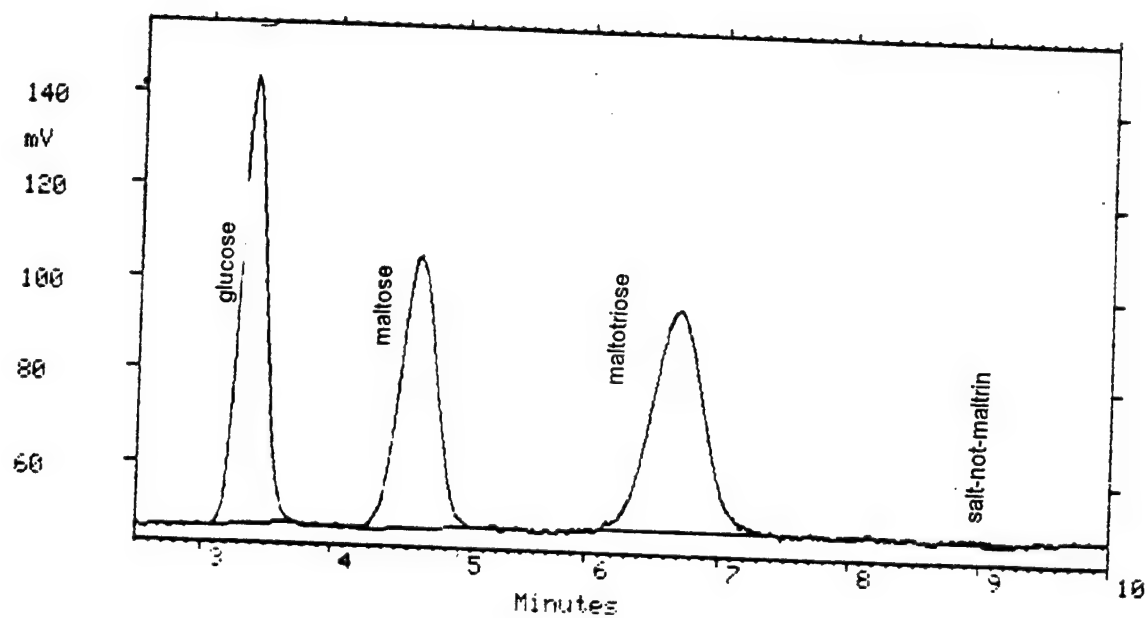
Figure 1. HPLC of glucose, maltose and maltotriose standards. Column: Waters Carbohydrate; Mobile phase: 70% acetonitrile-30% water; Flow rate: 2 mL/min; Detection: Refractive Index.
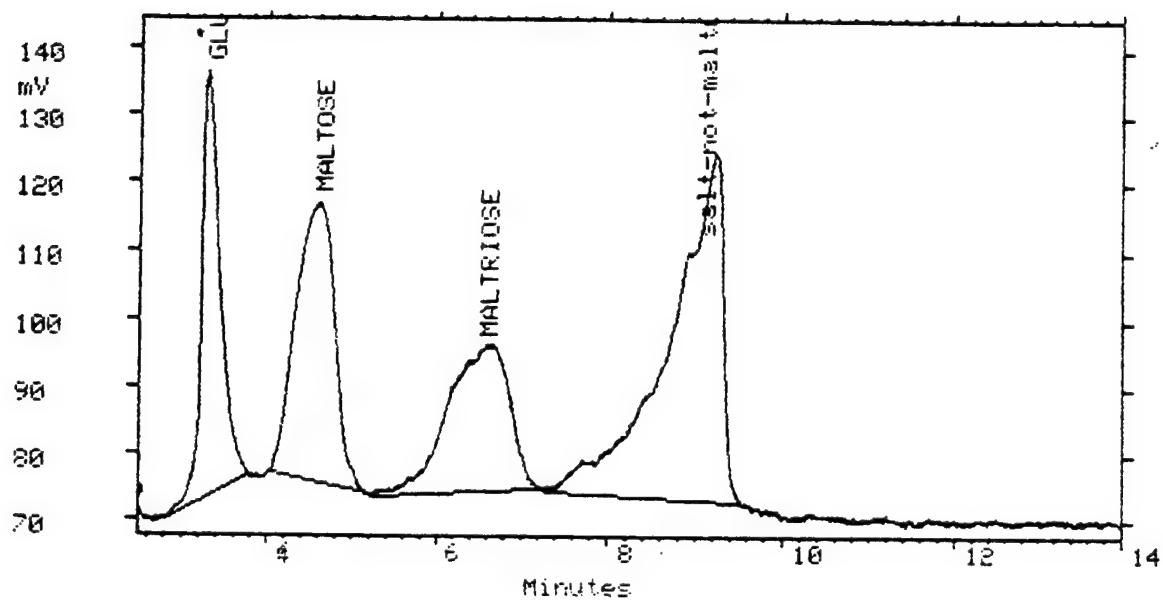
Figure 2. HPLC of α-amylase digest of pouch bread (PBRD). See Fig. 1 for details.
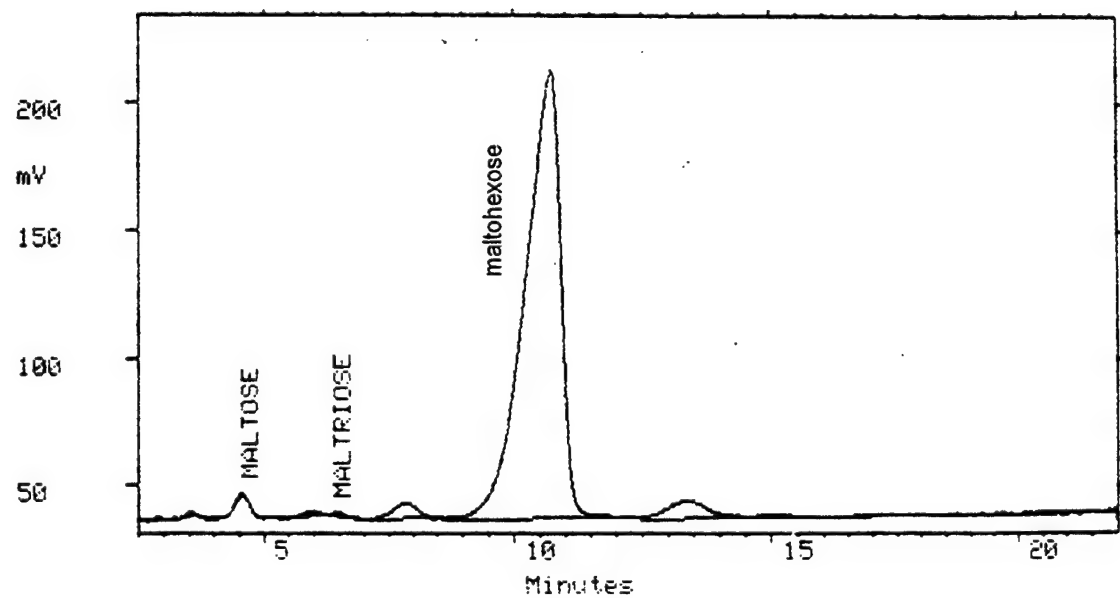
Figure 3. Retention of maltohexose and maltoheptose on Carbohydrate column using 70% acetonitrile.
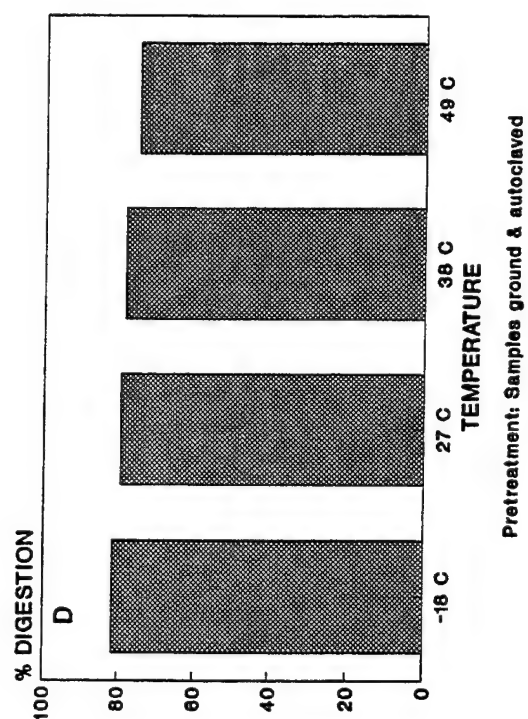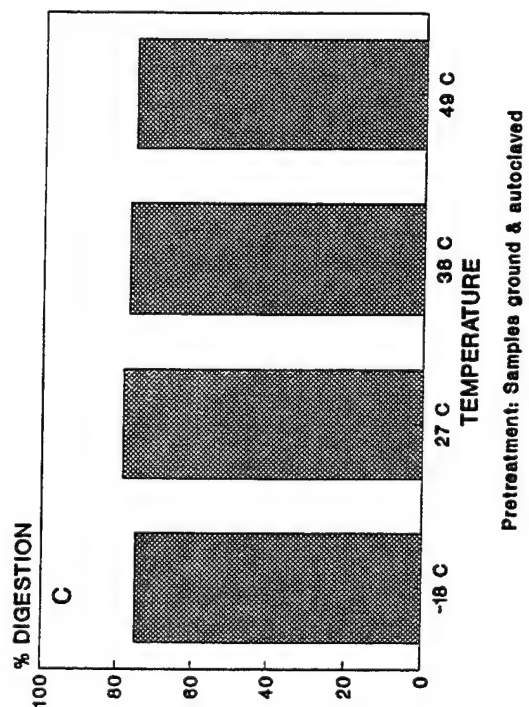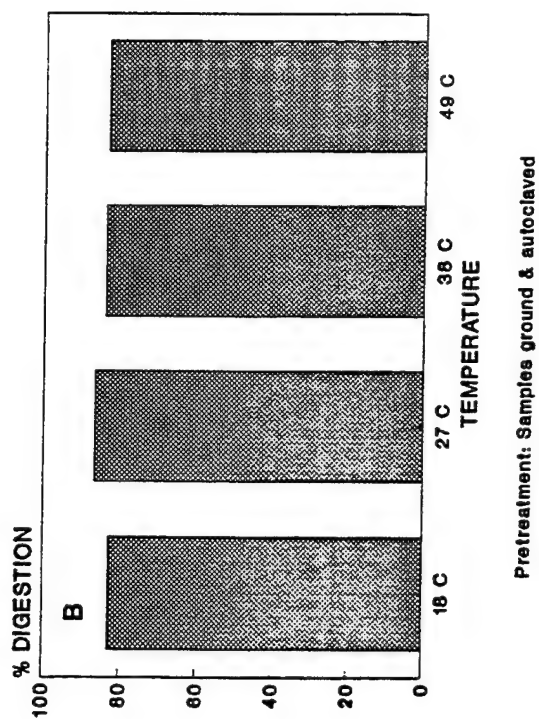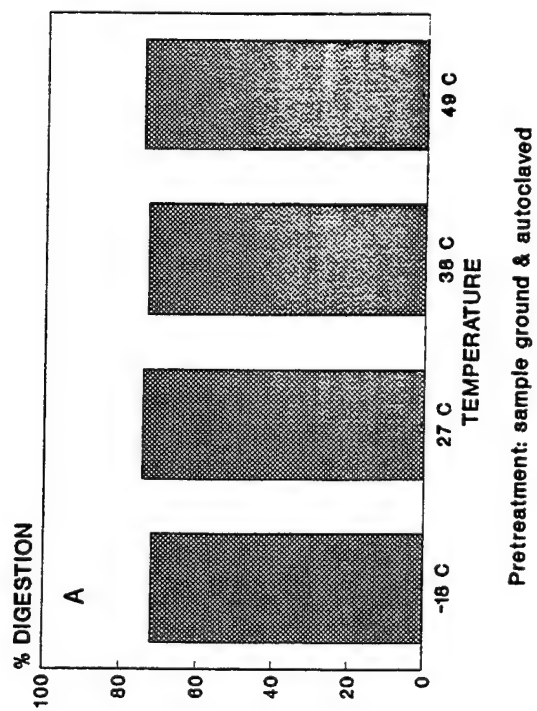
Figure 4. Digestibility by α-amylase of PBRD stored at -18° to 49° C. A) 1; B) 3; C) 6; & D) 12months. HPLC data.

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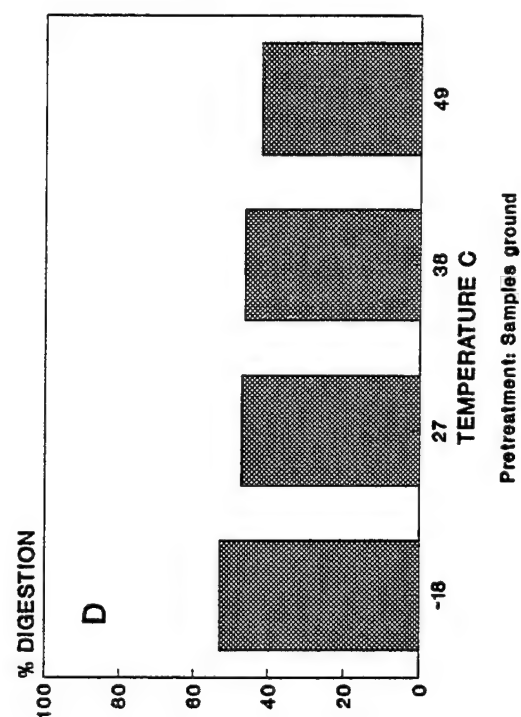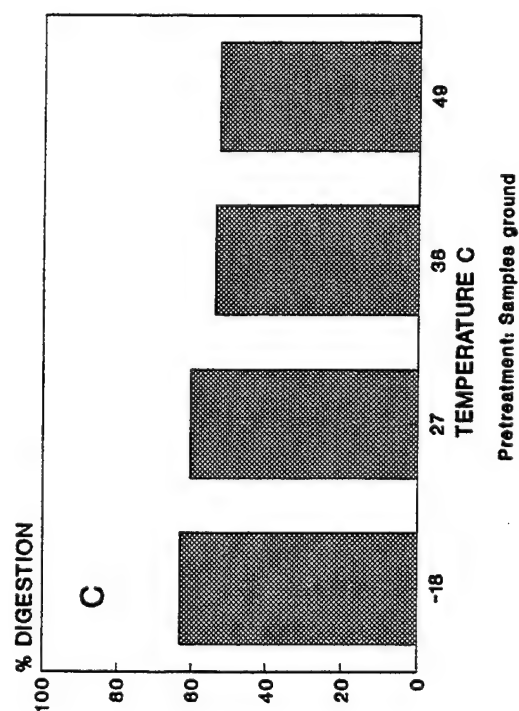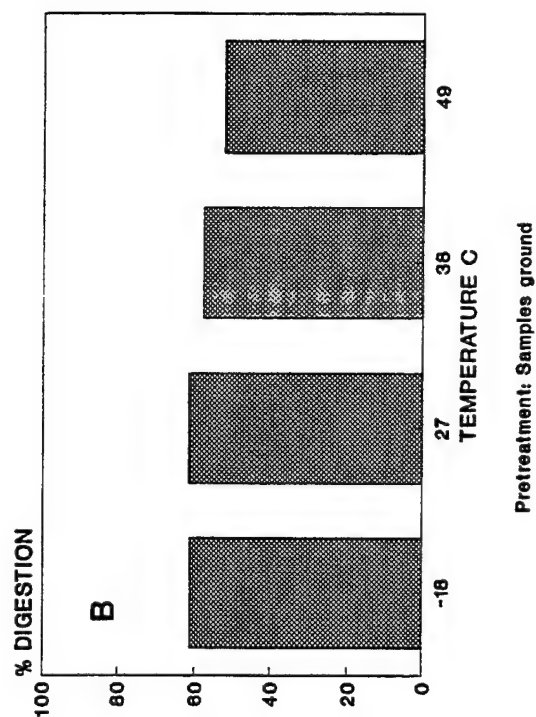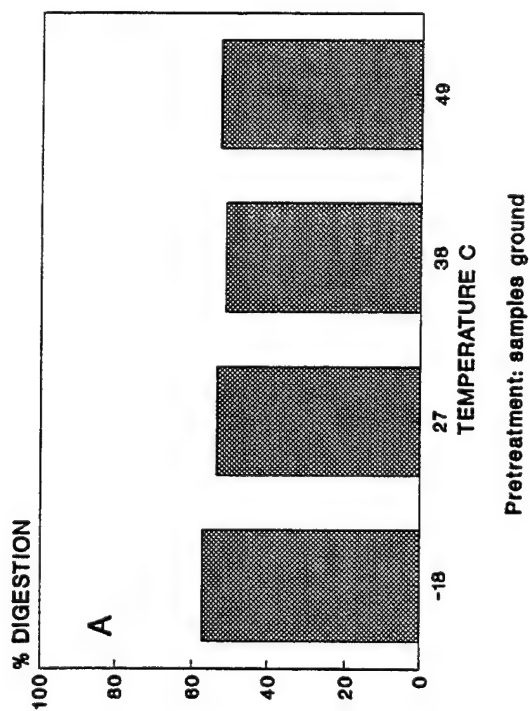
Figure 5. Digestibility by α-amylase of PBRD stored at -18° to 49° C. A) 1; B) 3; C) 6; D) 12 months. HPLC data.
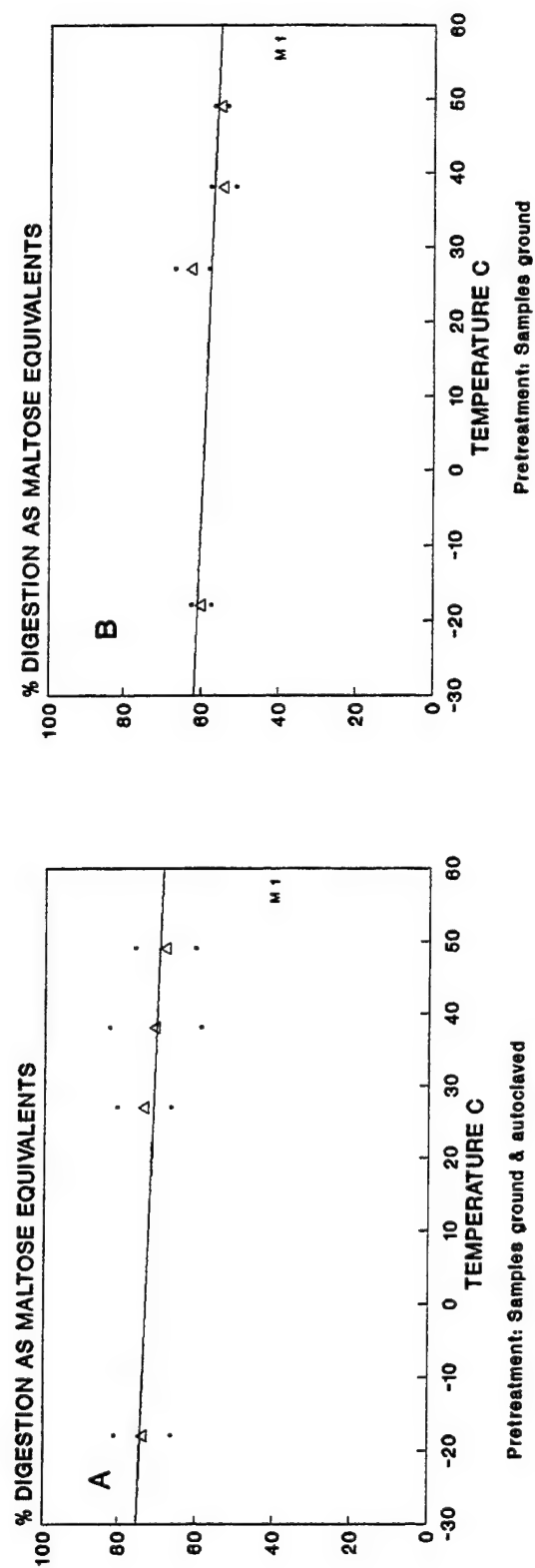
Figure 6. Digestibility by α-amylase of PBRD stored for 1 Month at -18° to 49° C. Chemical analysis data.

32
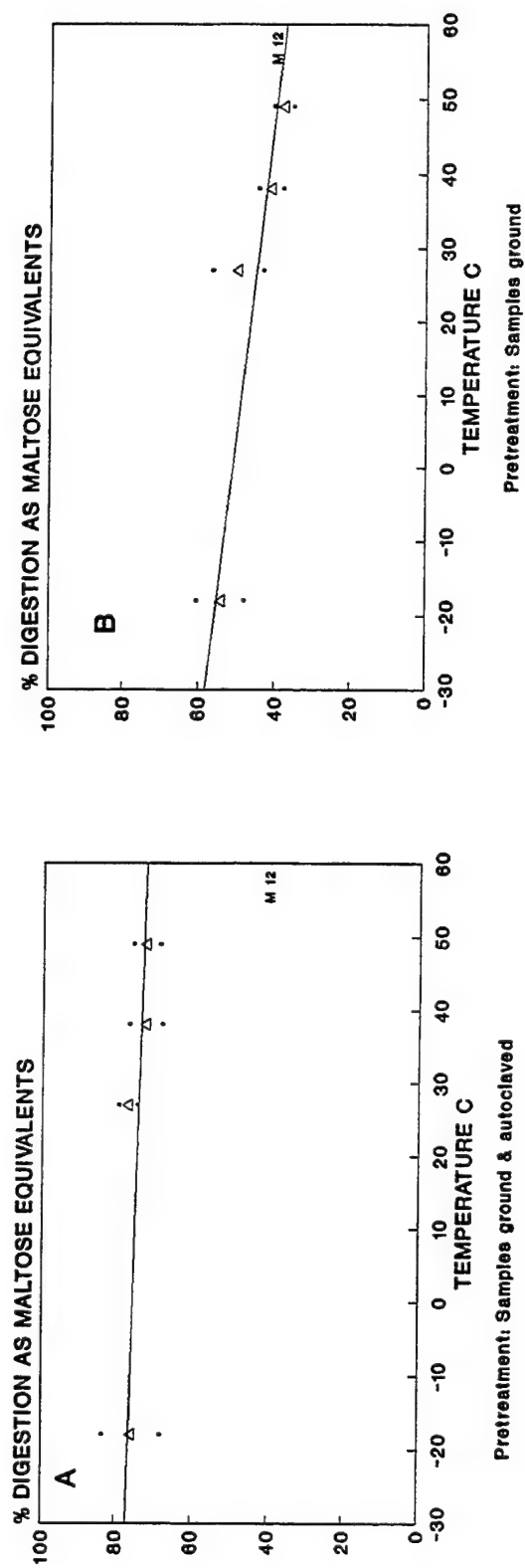
Figure 7. Digestibility by α-amylase of PBRD stored 12 Months at -18° to 49° C. Chemical analysis data.
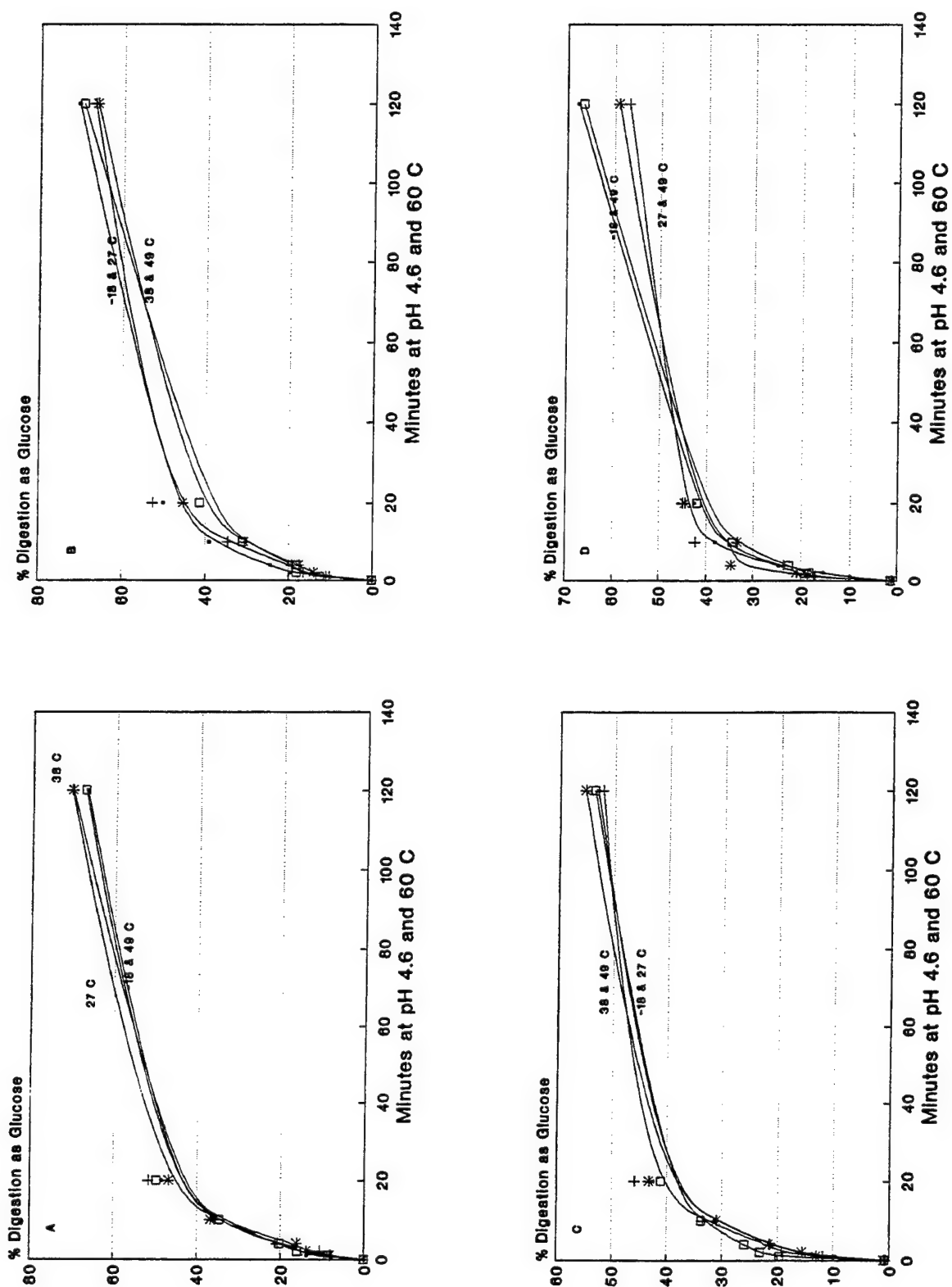
Figure 8. Rate of digestion of PBRD by amyloglucosidase during storage at -18° to 49° C. A) 1; B) 3; C) 6; & D) 12 months.

34
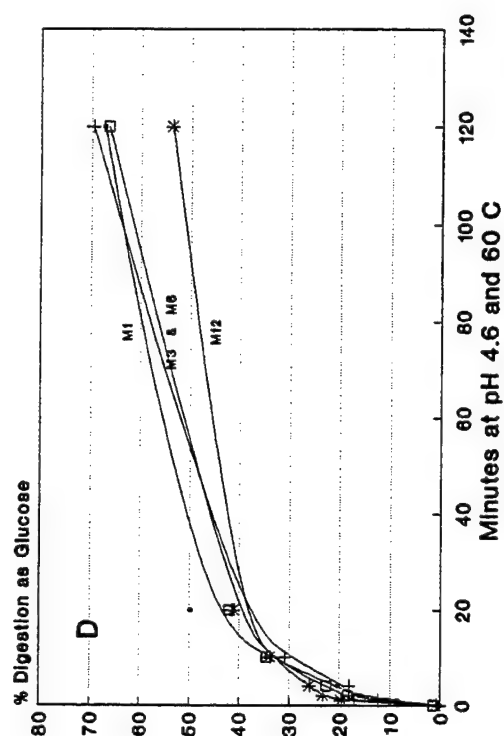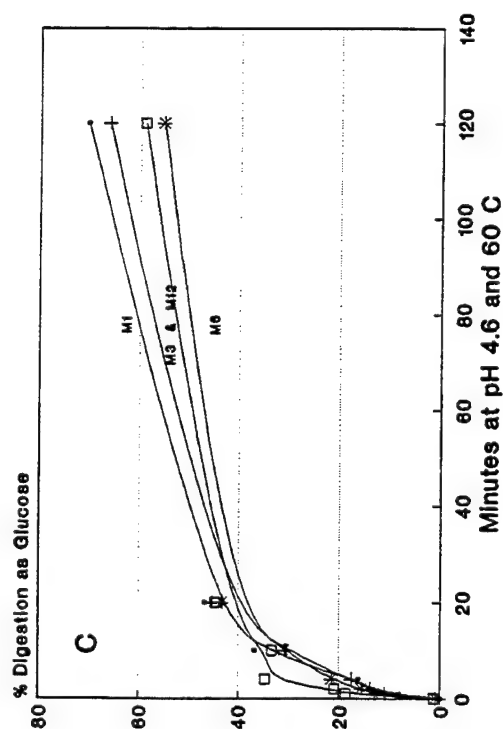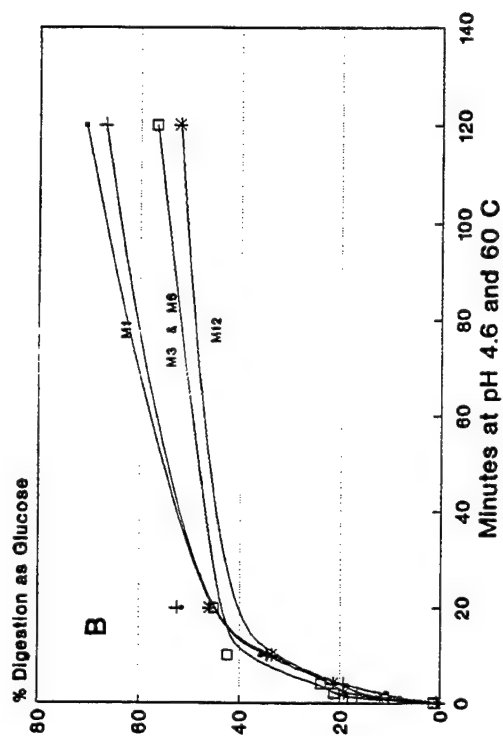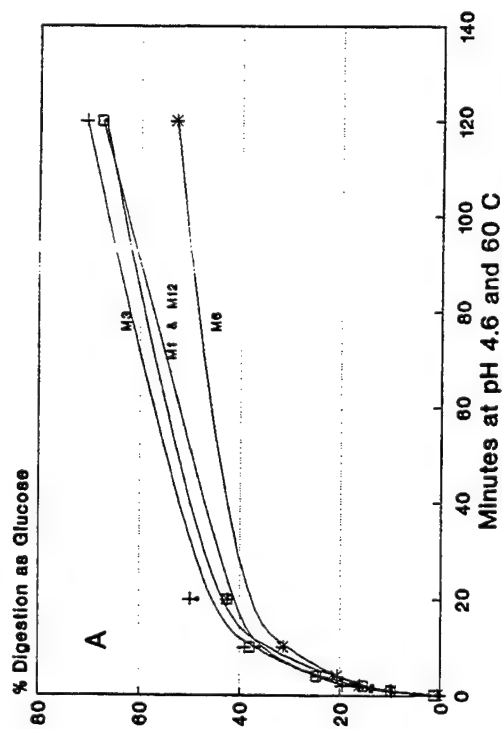
Figure 9. Rate of digestion of PBRD by amyloglucosidase during storage 1 to 12 Months. A) -18° C; B) 27° C; C) 38° C; & D) 49° C.

35
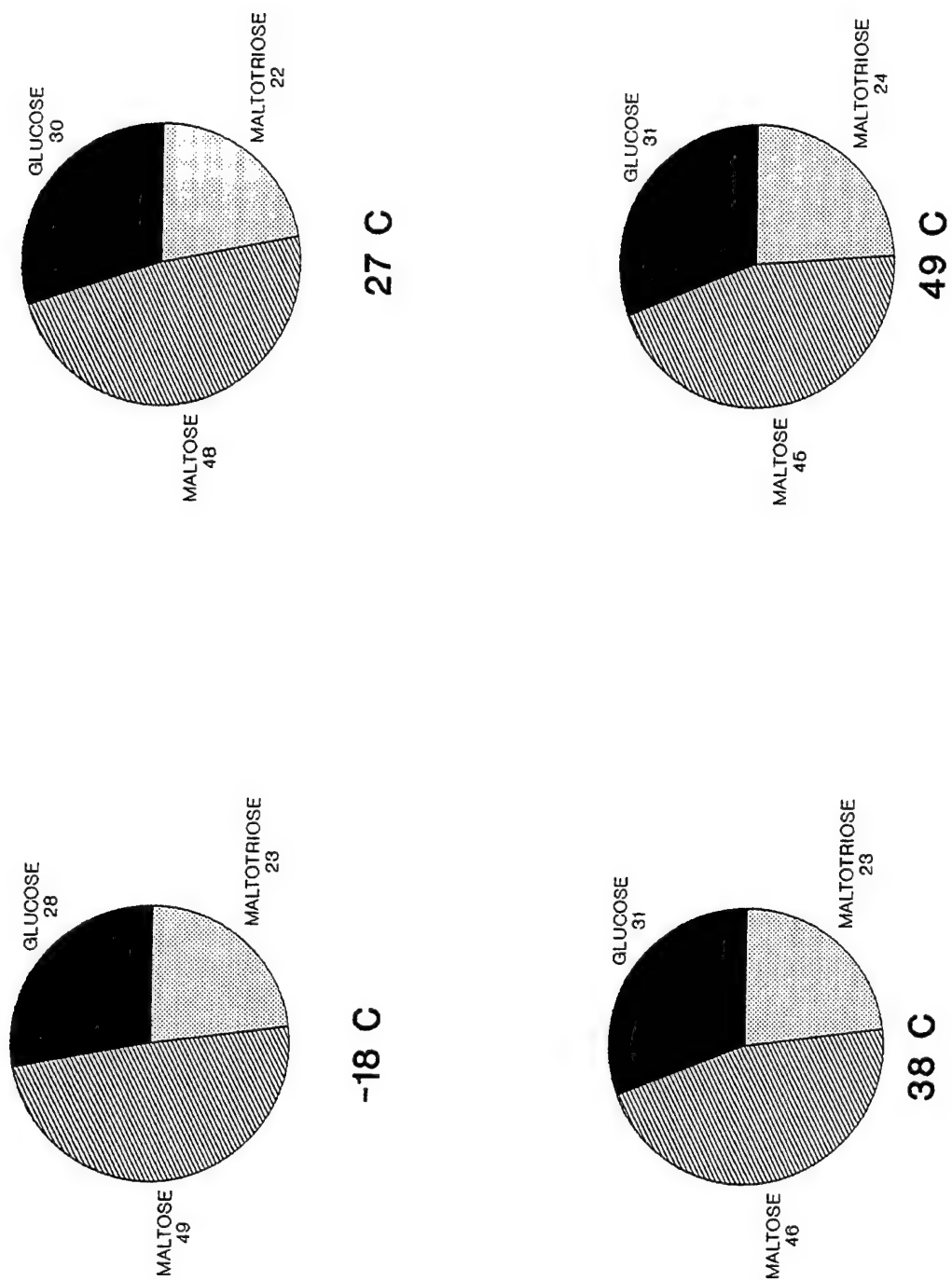
Figure 10. Composition of amylase digested carbohydrates in PBRD stored for 1 month at -18° to 49° C. Pretreatment: samples ground.

GLUCOSE
20

MALTOTRIOSE
33

MALTOSE
47

27 C

GLUCOSE
23

MALTOTRIOSE
32

MALTOSE
45

49 C

GLUCOSE
18

MALTOTRIOSE
33

MALTOSE
49

-18 C

GLUCOSE
21

MALTOTRIOSE
32

MALTOSE
47

38 C
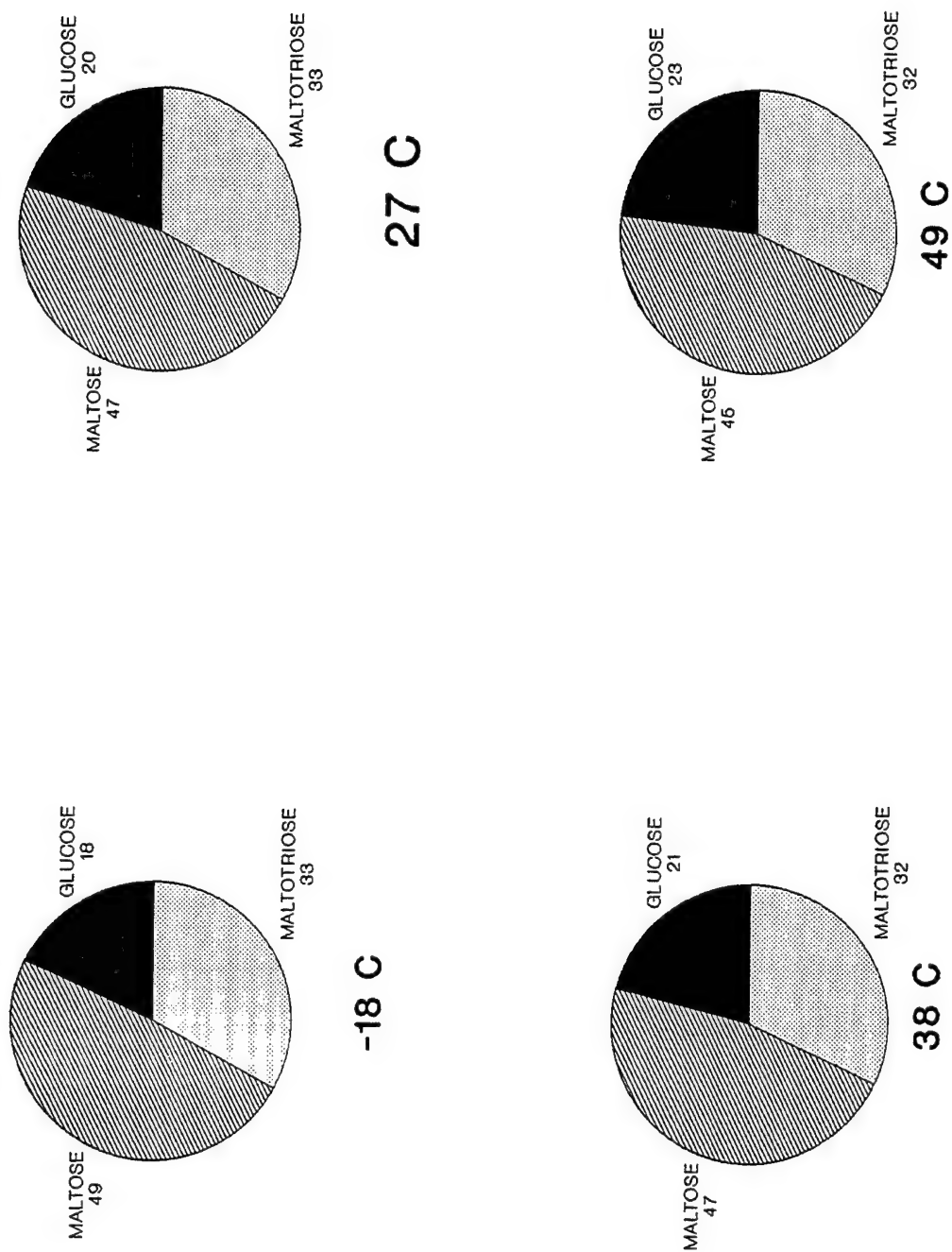
Figure 11. Composition of amylase digested carbohydrates in PBRD stored for 1 month at -18° to 49° C. Pretreatment: samples ground and autoclaved.
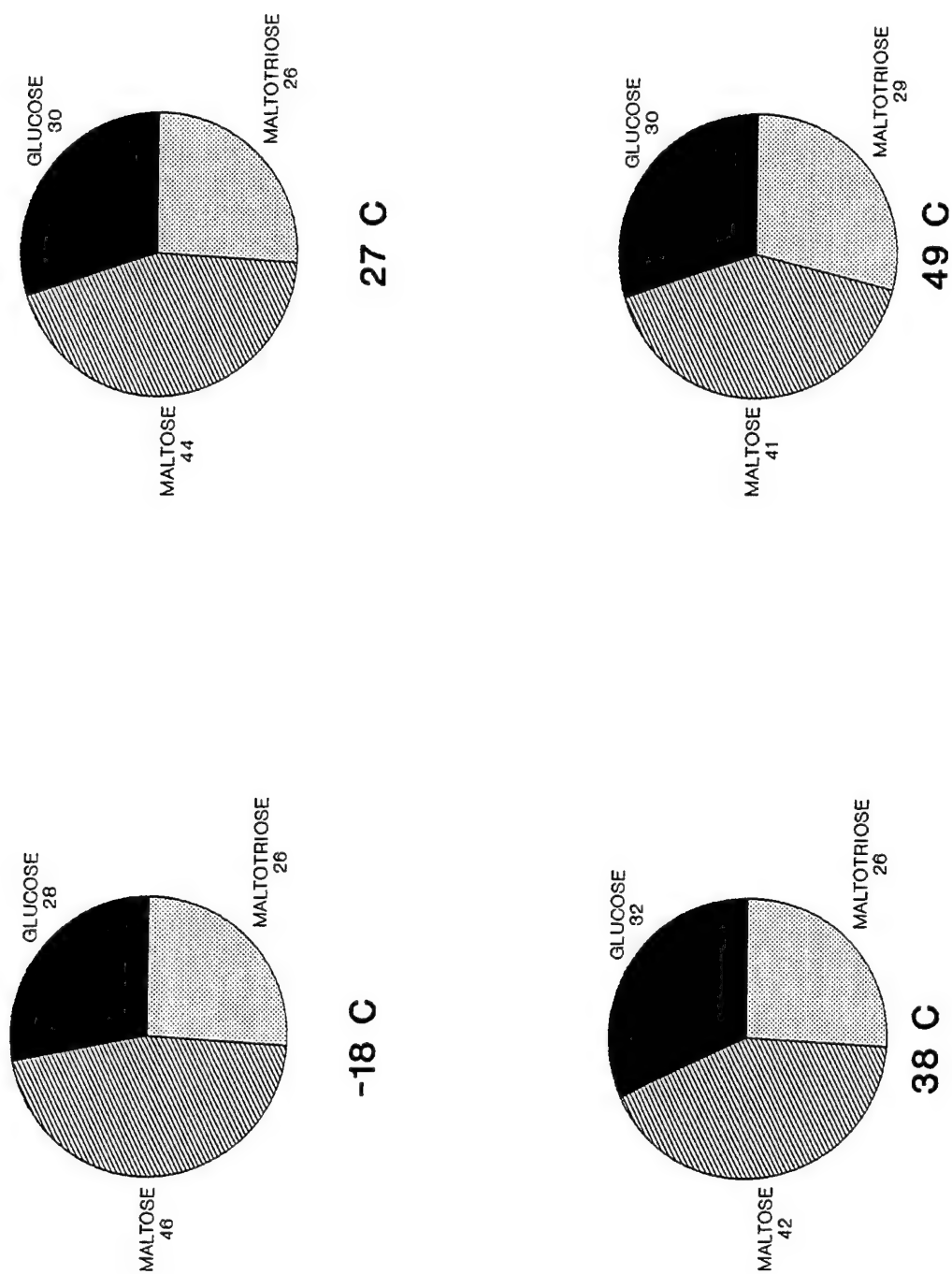
Figure 12. Composition of amylase digested carbohydrates in PBRD stored for 3 month at -18° to 49° C. Pretreatment: samples ground.
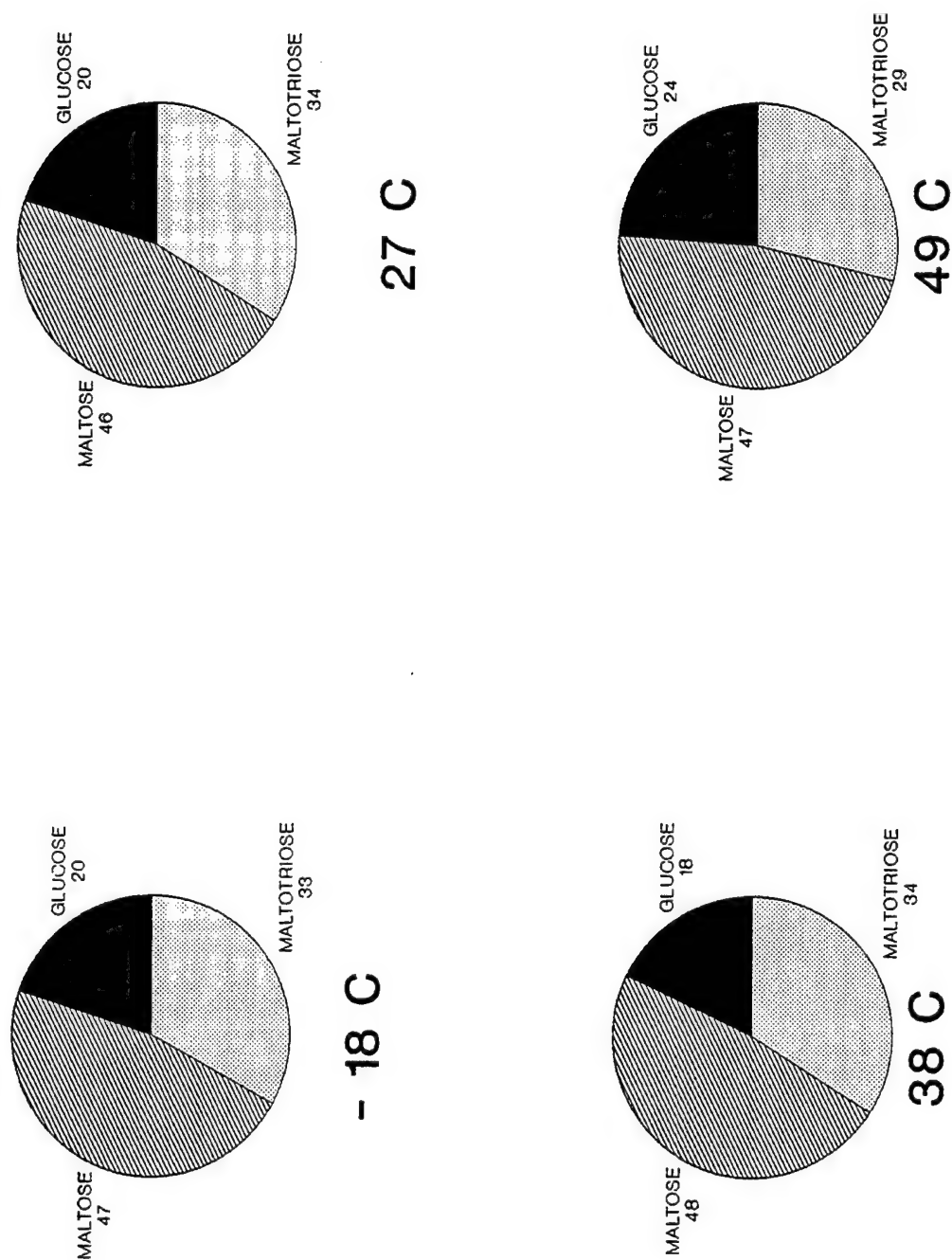
Figure 13. Composition of amylase digested carbohydrates in PBRD stored for 3 month at -18° to 49° C. Pretreatment: samples ground and autoclaved.
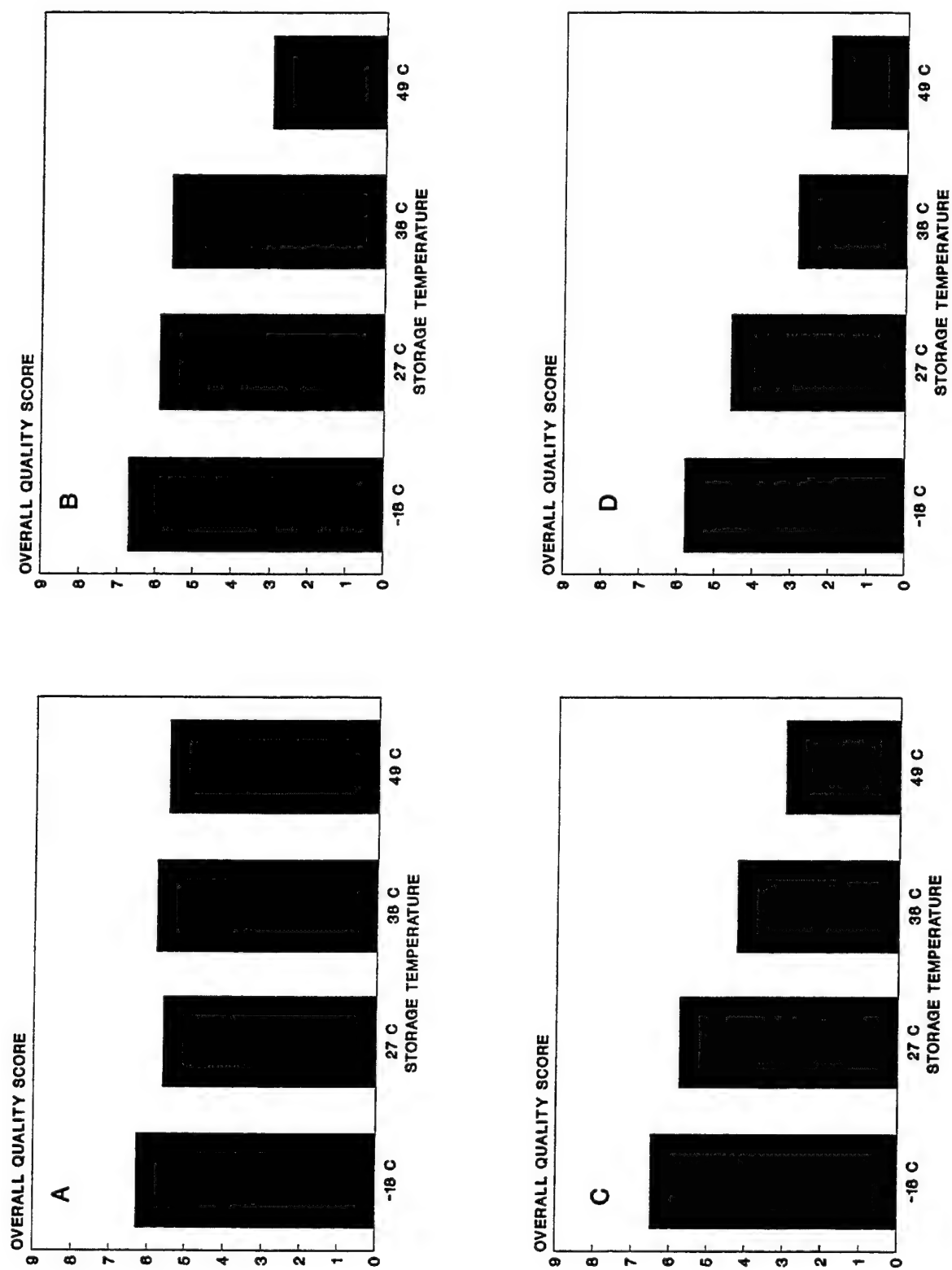
Figure 14. Sensory overall quality scores of PBRD stored at -18° to 49° C. A) 1; B) 3; C) 6 & D) 12 months.
1 = Extremely poor; 9 = Excellent.

40
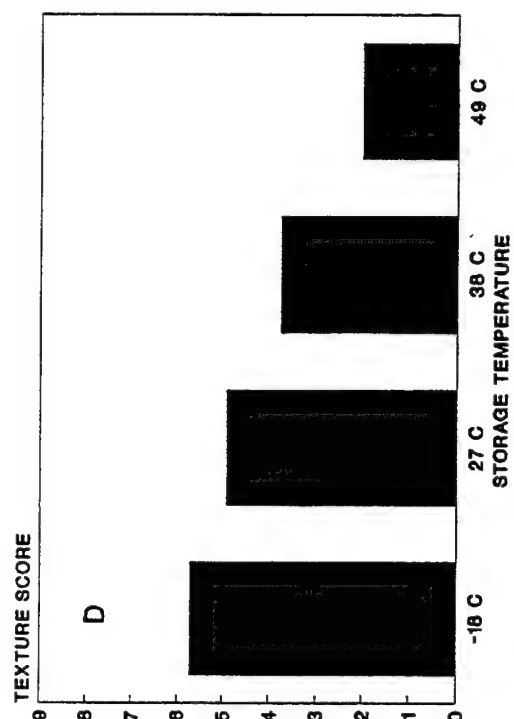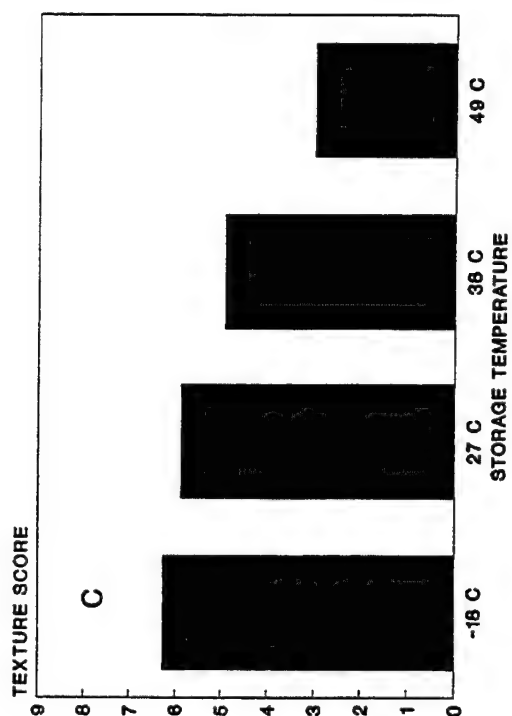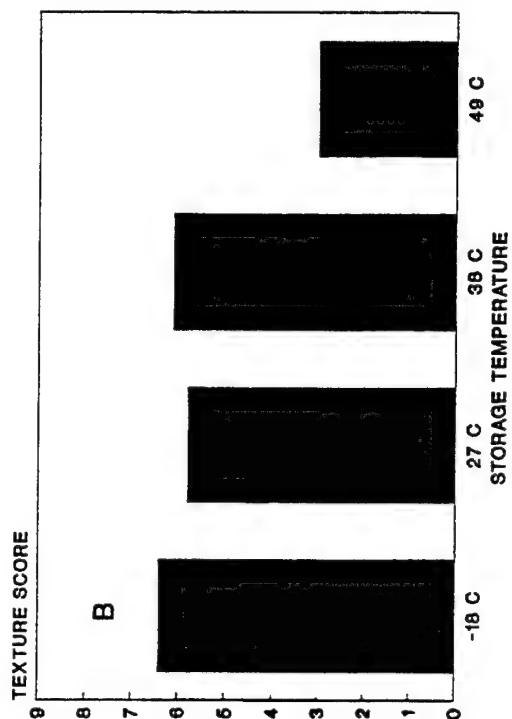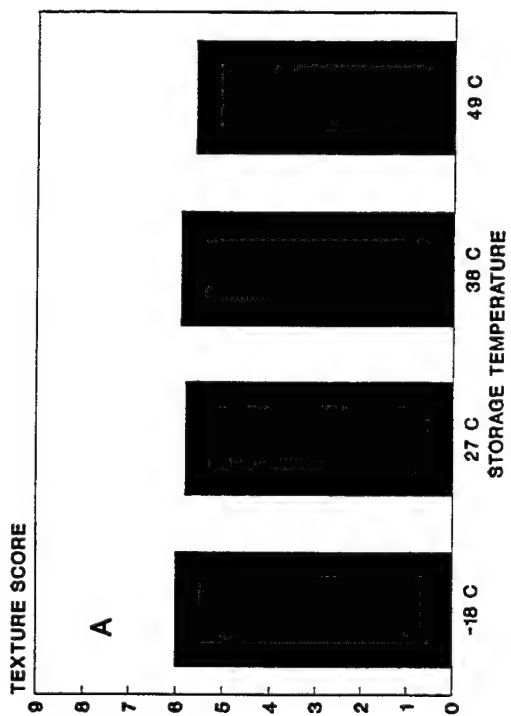
Figure 15. Sensory texture scores of PBRD stored at -18° to 49° C. A) 1; B) 3; C) 6 & D) 12 months.
1 = Extremely poor; 9 = Excellent.

OVERALL QUALITY SCORE
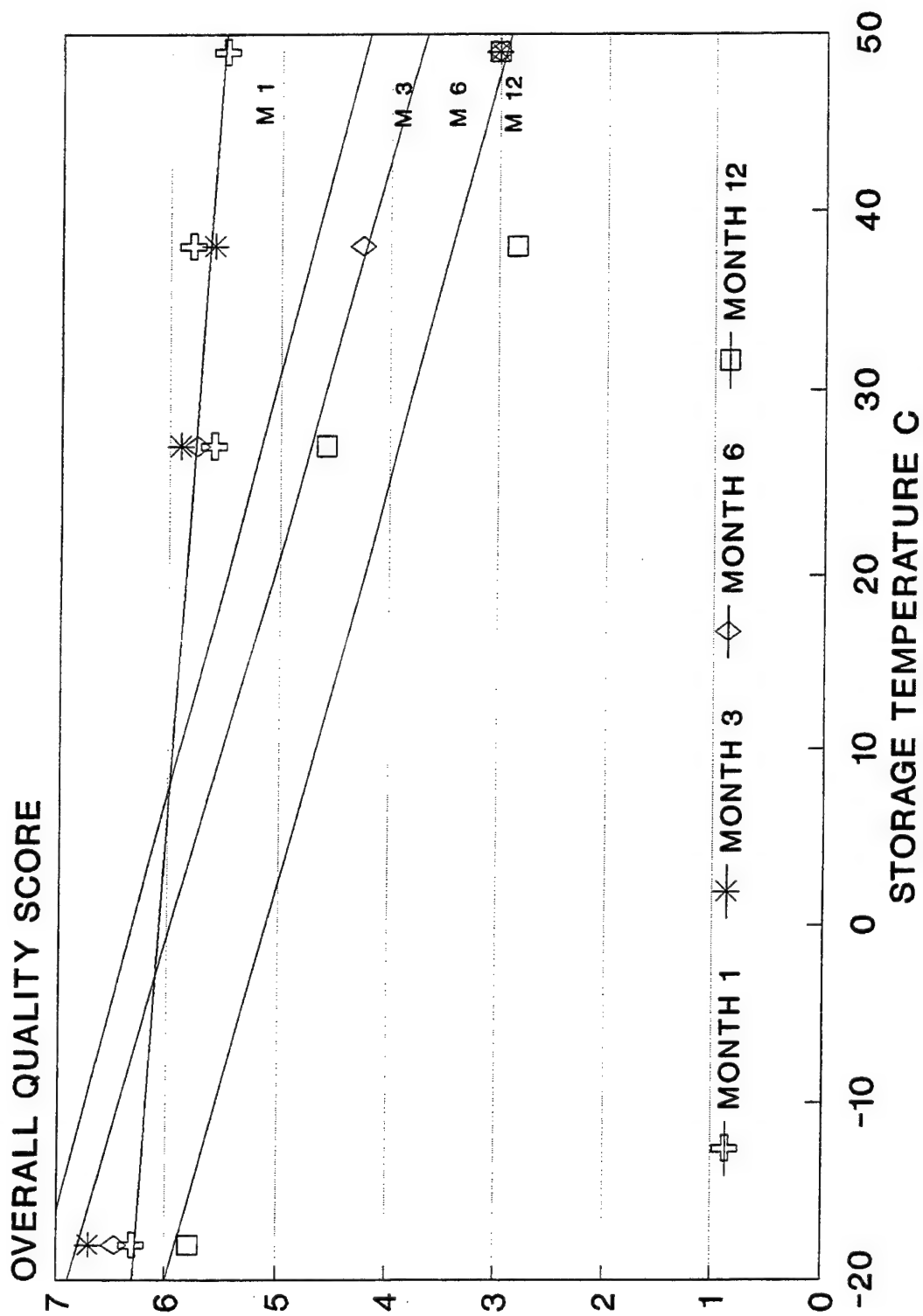
Figure 16. Regression plots. Sensory overall quality of PBRD stored 1 to 12 months vs. Storage temperatures of -18° to 49° C. 1 = Extremely poor; 9 = Excellent.
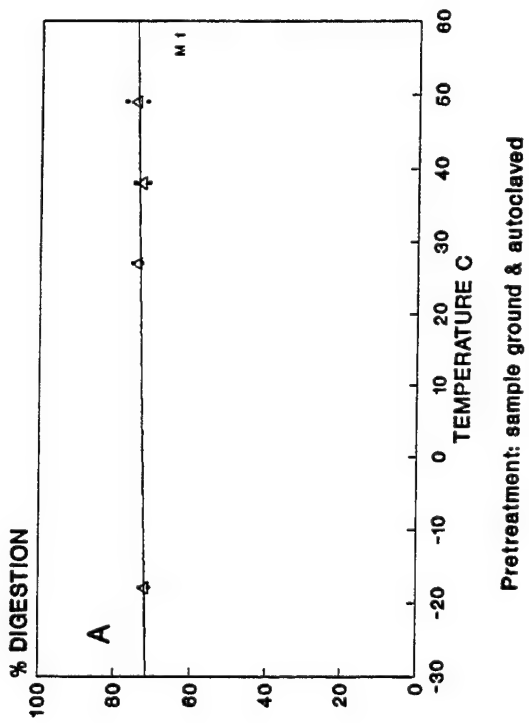
42
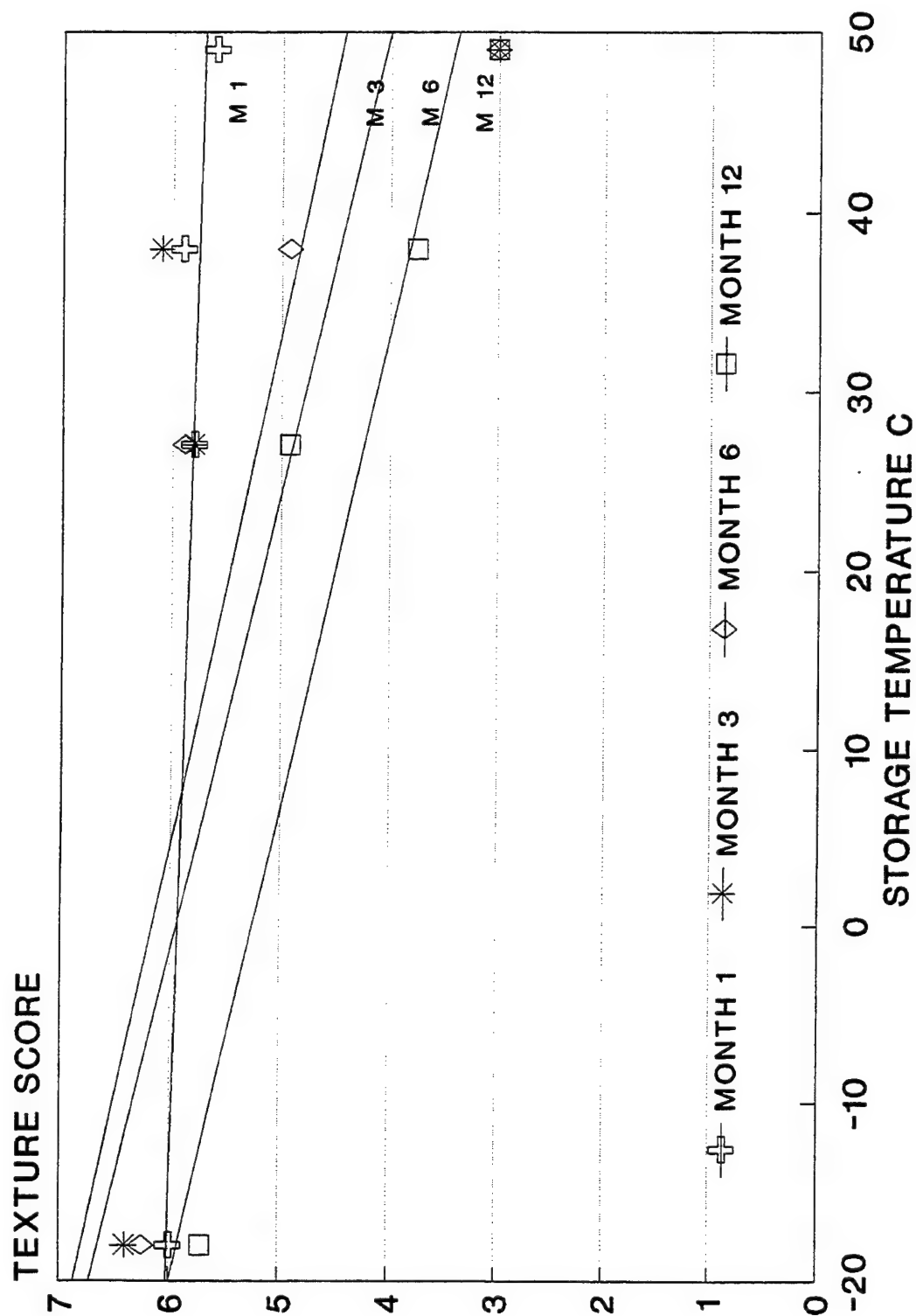
Figure 17. Regression plots. Sensory texture of PBRD stored 1 to 12 months vs. Storage temperatures of -18° to 49° C. 1 = Extremely poor; 9 = Excellent.
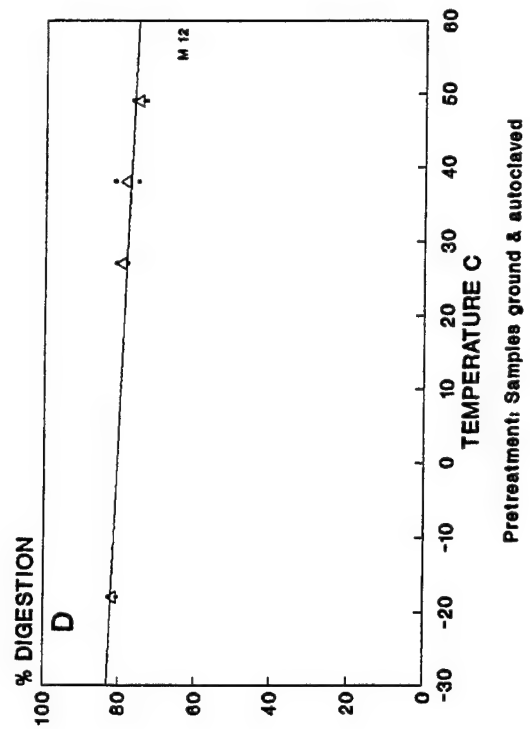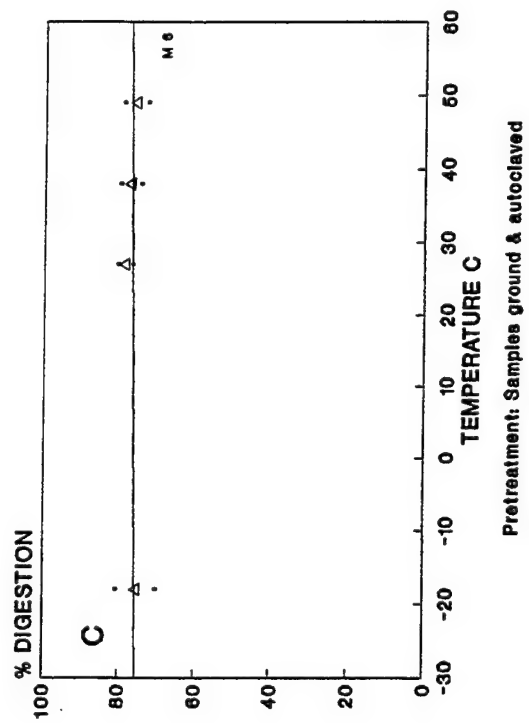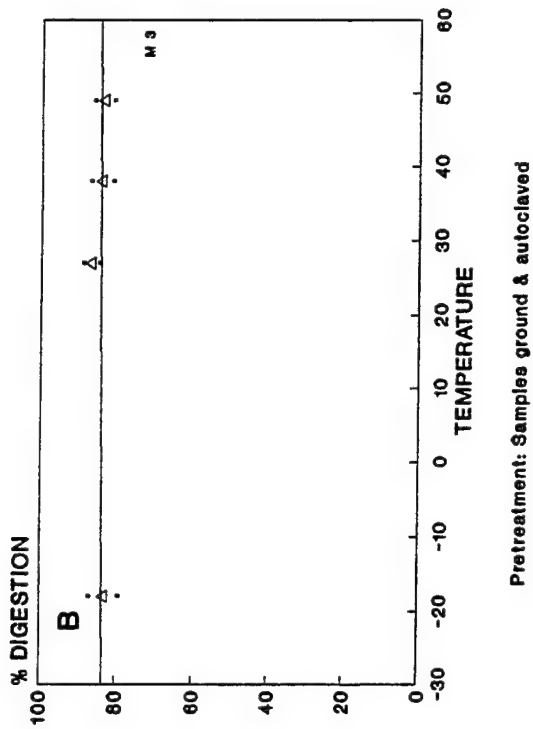
Figure 18. Regression plots. Percent digestion of PBRD vs. Storage temperatures of -18° to 49° C.
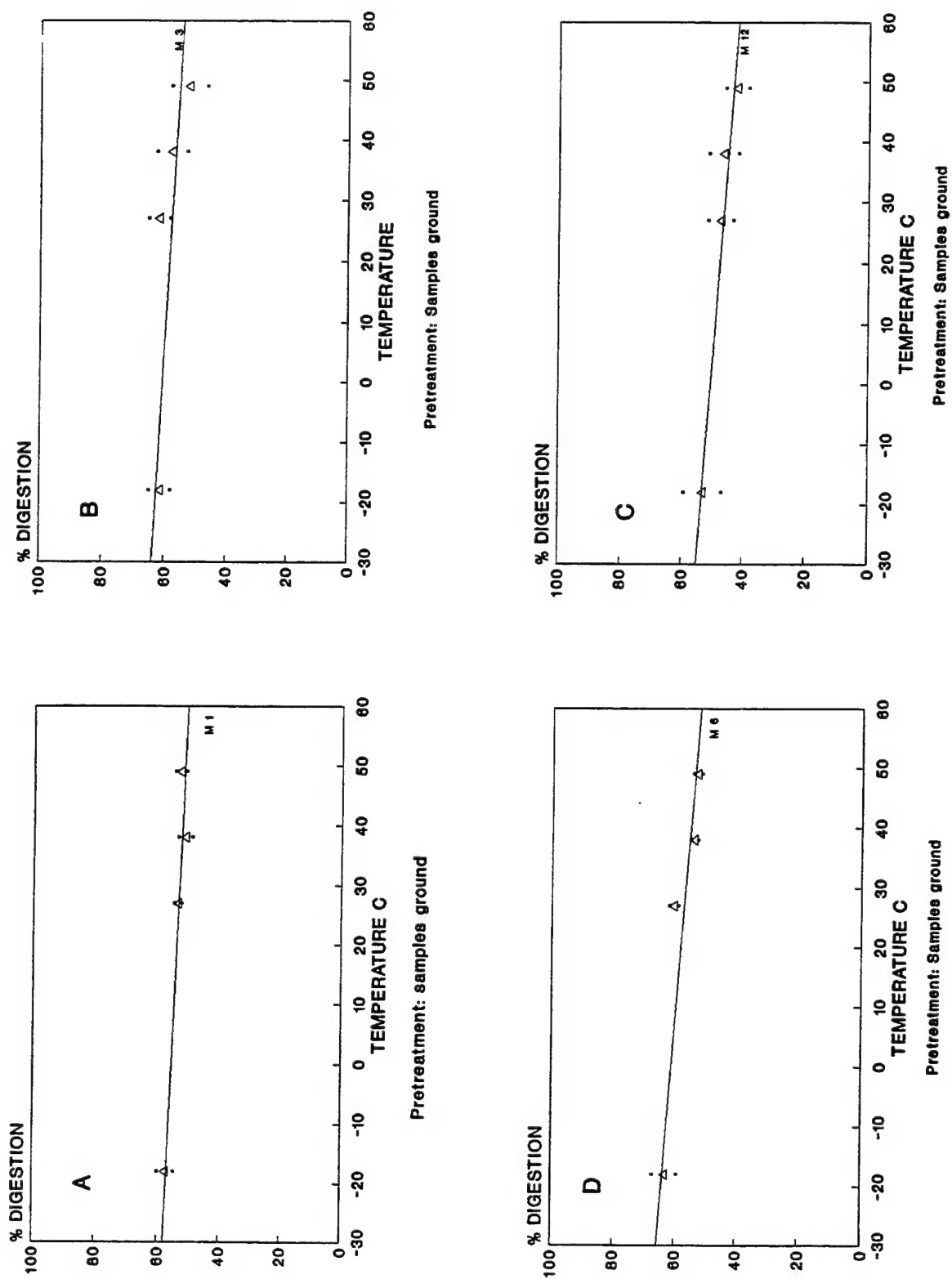A) 1; B) 3; C) 6; & D) 12 months.

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Figure 19. Regression plots. Percent digestion of PBRD *vs.* Storage temperatures of -18° to 49° C.
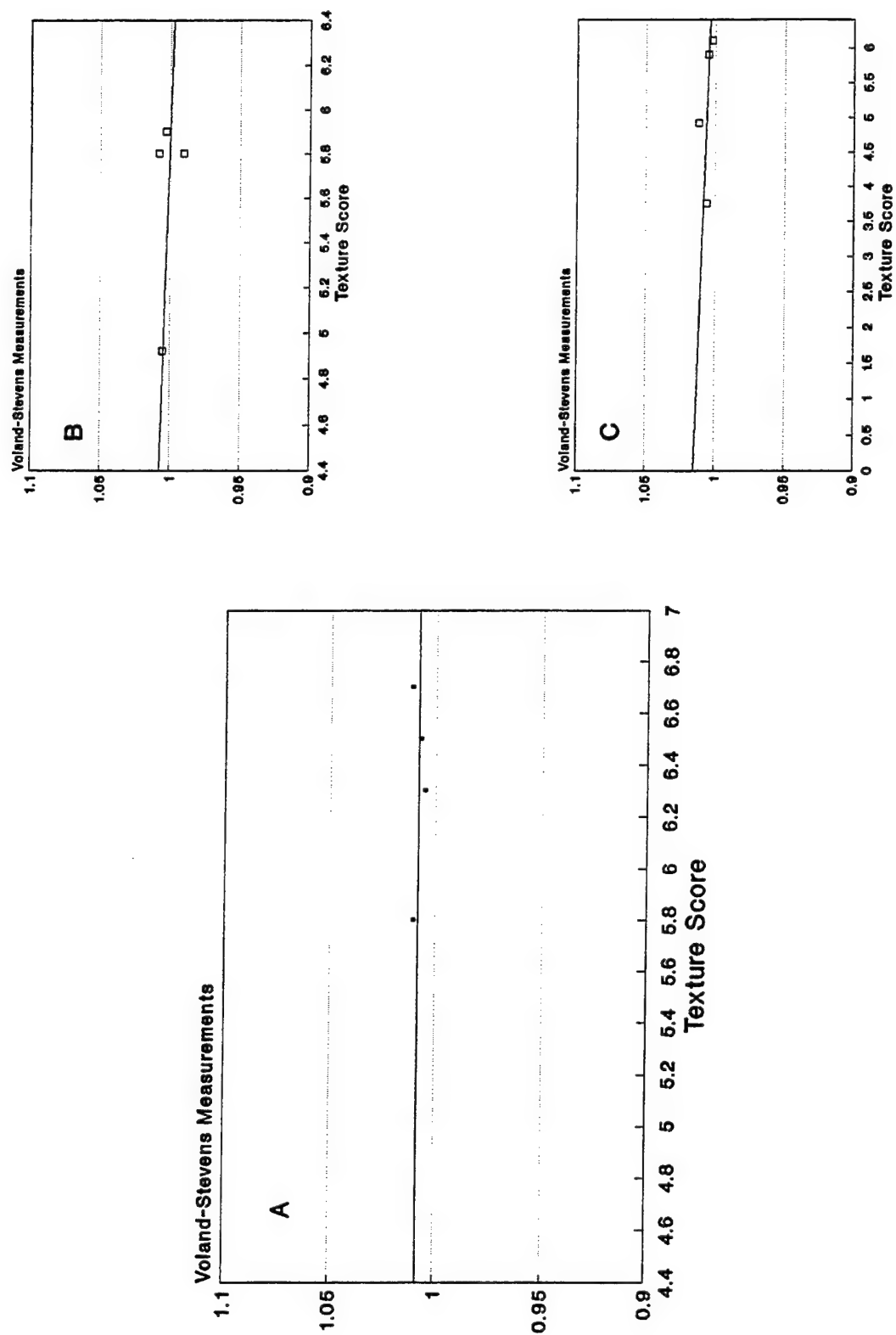A) 1; B) 3; C) 6; & D) 12 months.

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Figure 20. Correlations. Sensory texture of PBRD stored 1 to 12 Months *vs.* Voland-Stevens texture measurements.
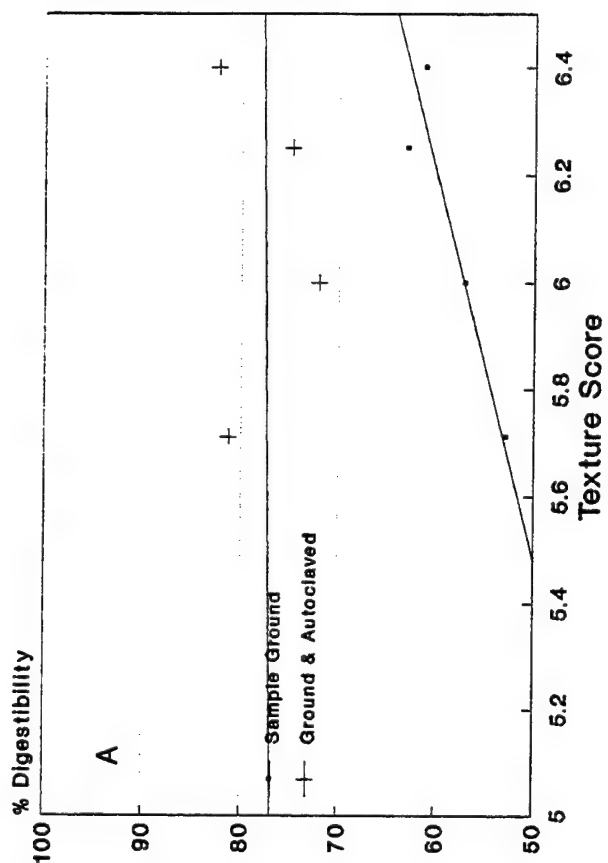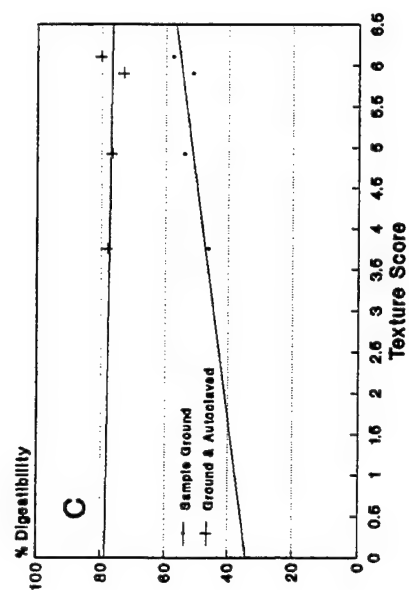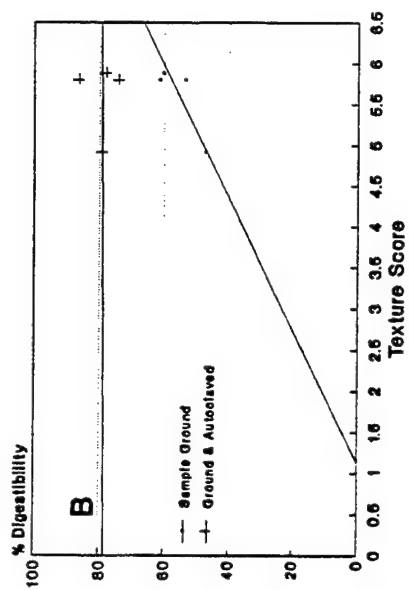A) -18° C; B) 27° C; C) 38° C

Figure 21. Correlations. Sensory texture scores vs. Amylase digestibility of PBRD stored 1 to 12 months.
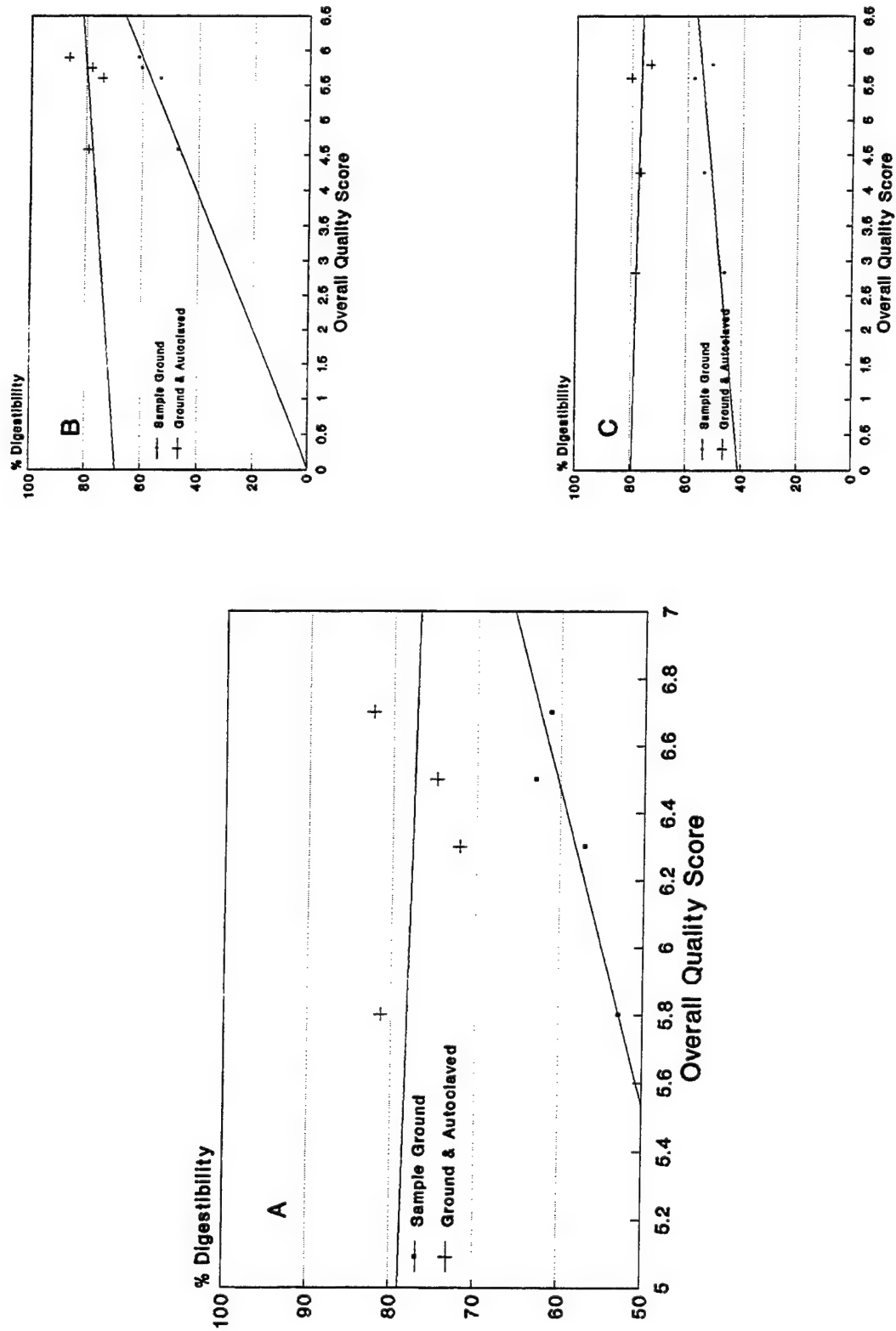A) -18°C; B) 27°C; & C) 38°C.

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Figure 22. Correlations. Sensory overall quality vs. Amylase digestibility of PBRD stored 1 to 12 months.
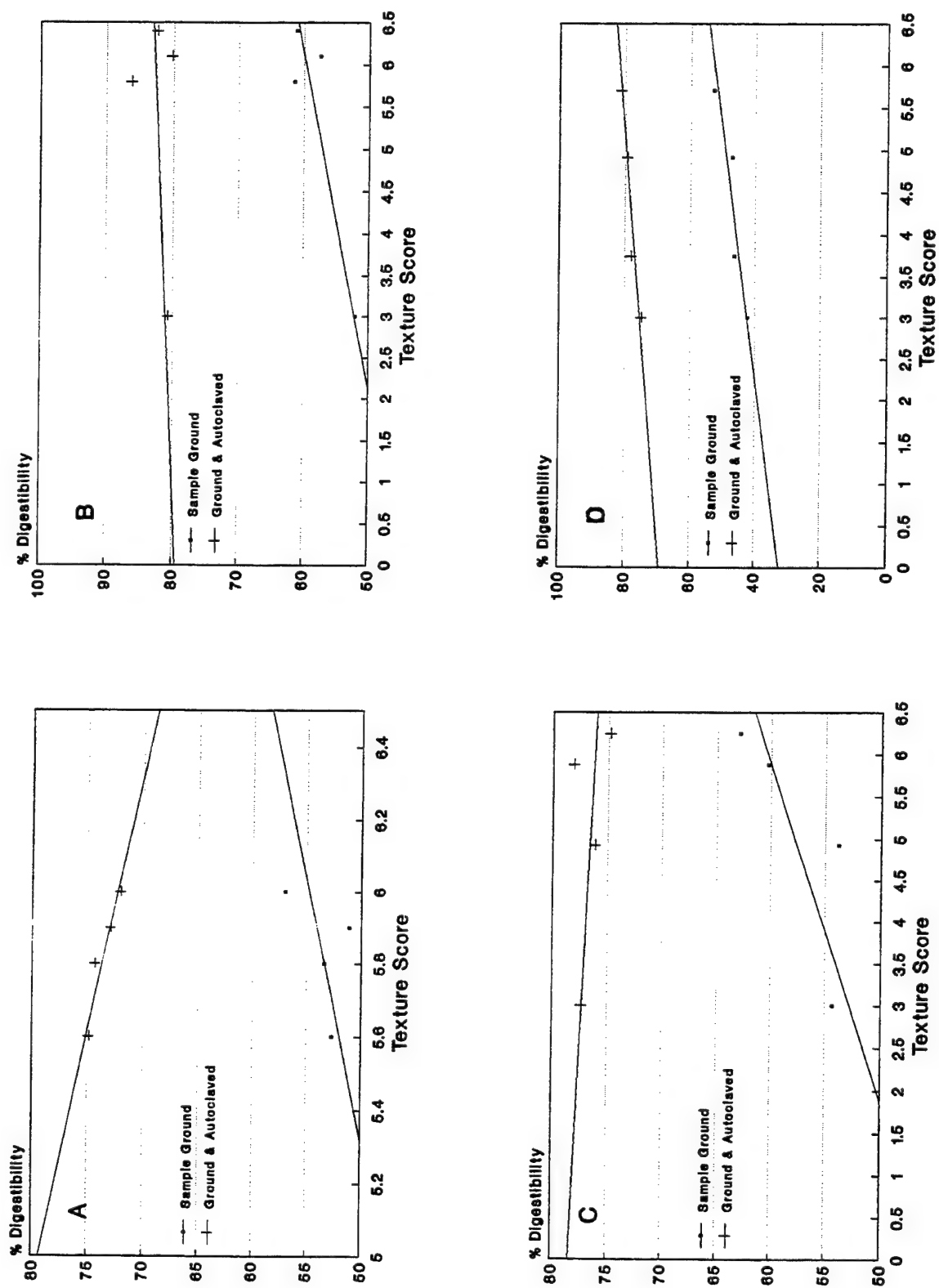A) -18°C; B) 27°C; & C) 38°C.

Figure 23. Correlations. Sensory texture scores vs. Amylase digestibility of PBRD stored -18° to 49° C.
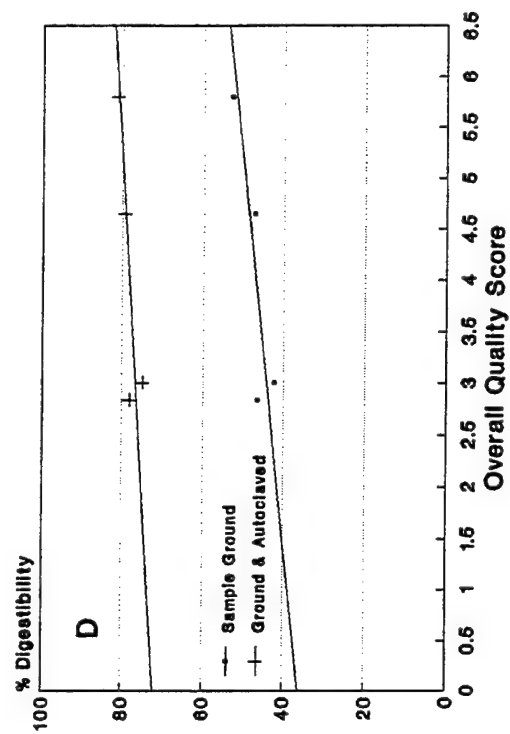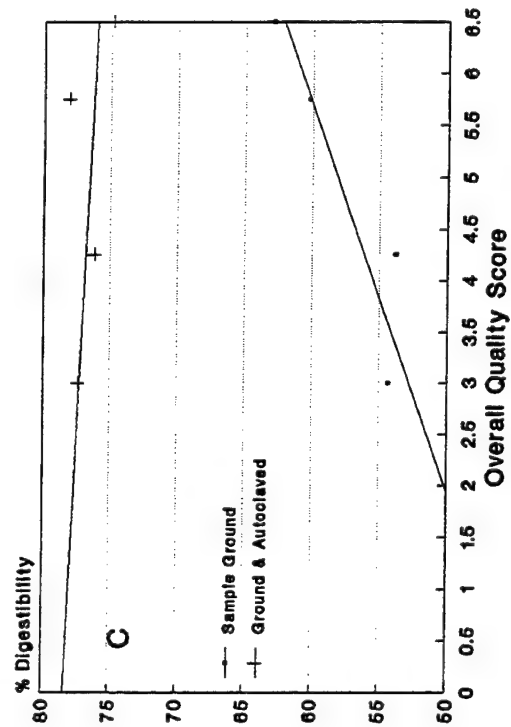A) 1; B) 3; & C) 6; & D) 12 months.
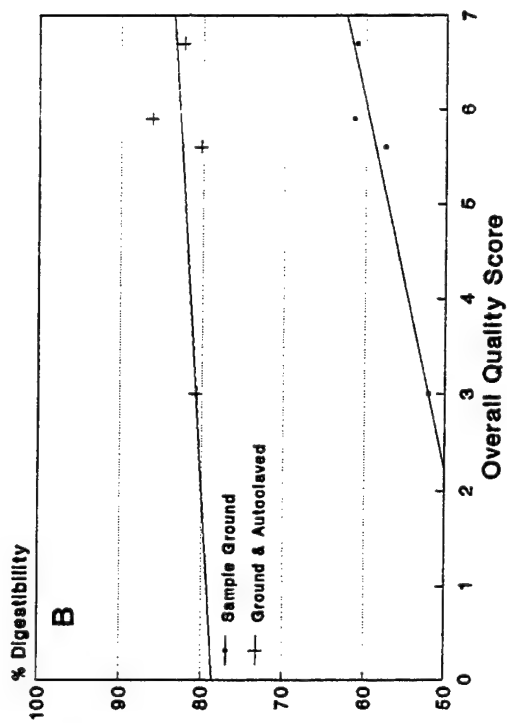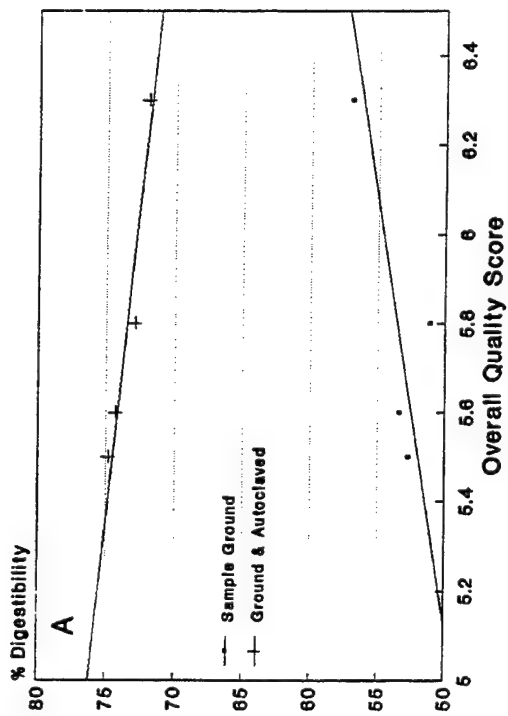
Figure 24. Correlations. Sensory overall quality vs. Amylase digestibility of PBRD stored -18° to 49° C.
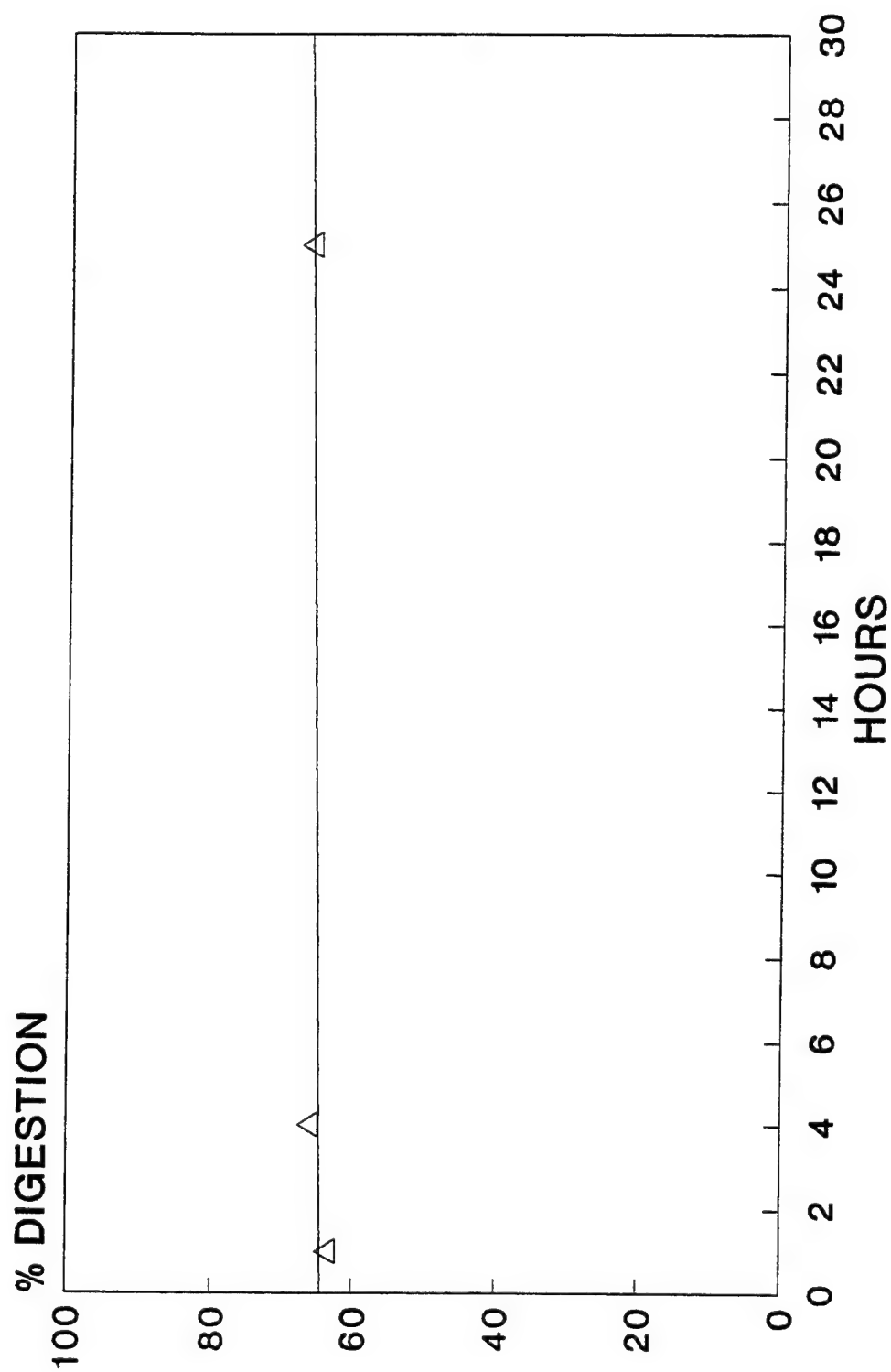A) 1; B) 3; & C) 6; & D) 12 months

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Figure 25. Digestibility of a commercial starch fiber by amylase during prolonged incubation at 37° C. Pretreatment: samples ground and autoclaved.
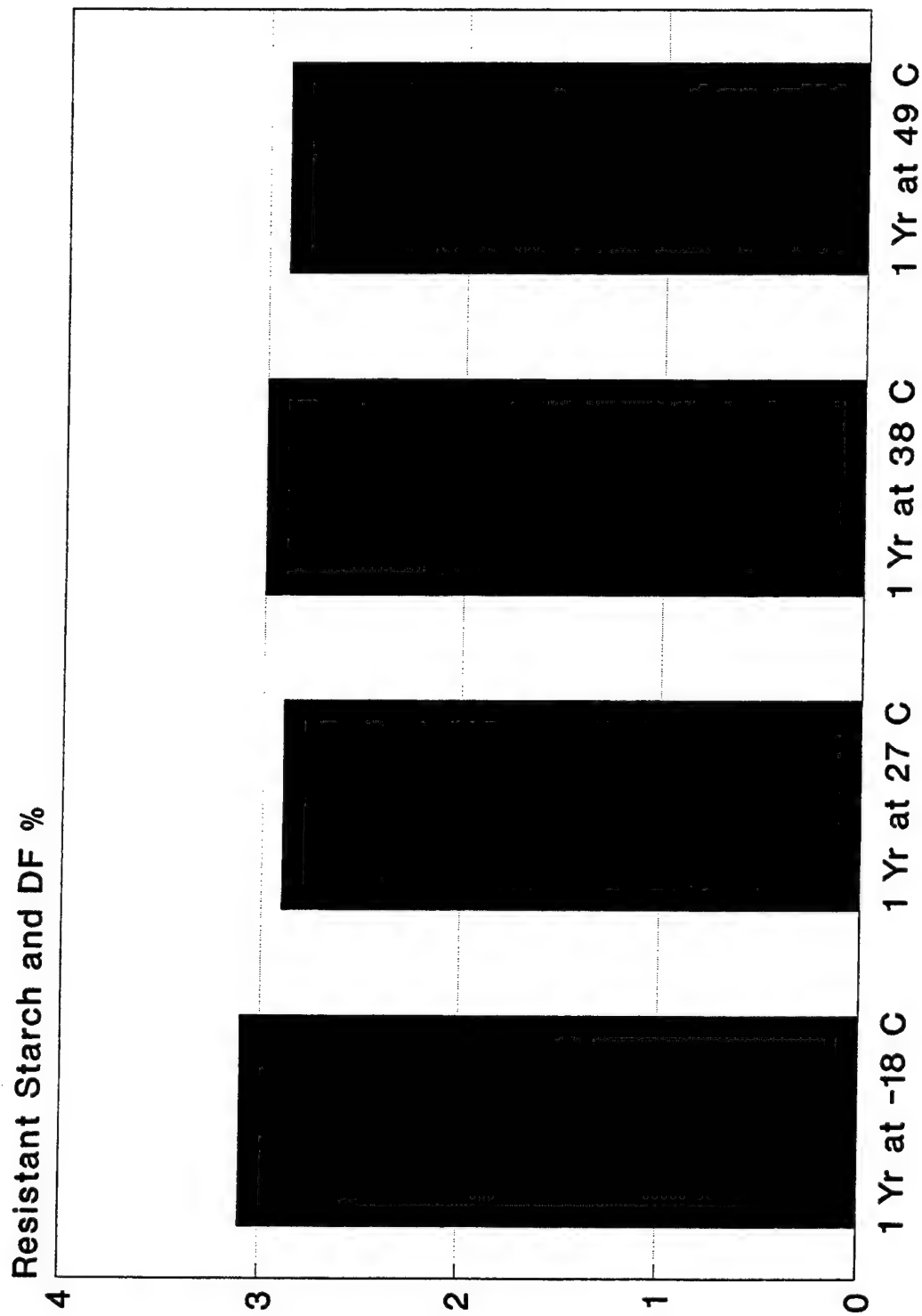
Figure 26. Total dietary fiber and resistant starch in 12 month PBRD stored at -18°, 27°, 38° & 49° C.

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